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EFFECTS OF THREE INTENSITIES OF MATERNAL EXERCISE ON THE MATERNAL RAT AND DEVELOPMENT OF THE FETUS

by

MICHELLE FRANCES MOTTOLA

(C)

A THESIS

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ABSTRACT

The purpose of this study was to observe the effects of three intensities of maternal aerobic exercise (mild, moderate and severe) throughout gestation on both mother and fetus in the rat. The mild intensity (MILD) consisted of a treadmill speed of 15 m/min, on a 10° incline for a duration of 60 minutes/day, 5 days/week. The moderate intensity (MOD) consisted of a treadmill speed of 30 m/min, on a 10° incline, for 60 minutes/day, 5 days/week and the severe intensity (SEV) consisted of a treadmill speed of 30 m/min, on a 10° incline for 120 minutes/day, 5 days/week. In all three intensity experiments (MILD, MOD and SEV) young female Sprague-Dawley rats (Biosciences - University of Alberta) of approximately 50 days of age were used for study. Prior to random assignment of the rats into appropriate groups, the rats were conditioned to run by a progressive two week treadmill exercise program. Following the two week program, the rats were paired by weight and selected to fulfill one of two main experimental conditions: a pregnant group that continued their respective running intensity program throughout gestation (pregnant runner, PR-MILD, PR-MOD, PR-SEV), or a pregnant group that did not continue the running program throughout pregnancy (pregnant control, PC-MILD, PC-MOD, PC-SEV). At birth the neonates born to the PR-MILD, PR-MOD or PR-SEV groups did not differ in neonatal body weight values, number/litter or total litter weight values when compared to controls, nor were superficial gross

abnormalities observed in the neonates born to the PR groups. The SEV intensity exercise did not alter neonatal organ weight values (brain, heart, liver, lung, kidney), nor neonatal skeletal muscle development (gastrocnemius, sternomastoid, diaphragm) when compared to control values. The MILD exercise intensity did not alter maternal body weight gain during pregnancy. The MOD and SEV exercise intensities, however, did cause significant reductions in maternal body weight gain ($p<0.05$) when compared to control values. After giving birth, the maternal rats in the SEV exercise experiment (PR-SEV and PC-SEV) were skinned, and each component, skin (subcutaneous fat and mammary tissue), and the remainder, were weighed and compared. The PR-SEV group had significantly less skin and remainder body components than the PC-SEV group ($p<0.05$). It is suggested that in maternal running rats that exercise throughout gestation, adaptive mechanisms develop to protect the fetus, but these mechanisms appear to occur at the expense of the maternal system, especially at the more severe exercise intensities.

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INTRODUCTION

Society today emphasizes the beneficial aspects of exercise and fitness. This attitude has progressively expanded to also include the pregnant woman within its influential realm, with many magazines and books available to the general public promoting exercise during pregnancy. Unfortunately, advice concerning maternal exercise is usually limited to vague generalizations to avoid excessive fatigue, which substitutes for valid scientific observations (Cumming and Belcastro, 1982). A review of the literature reveals little conclusive information concerned with the effects of exercise on the pregnant woman and/or the developing fetus. To substantiate guidelines for women who wish to exercise during pregnancy, more scientific observations are needed in human research as well as observations through the use of animal models. From a basic overview of exercise physiology it would seem reasonable to suggest that there are several immediate areas of interest which may be related to the effects of exercise performed by the pregnant woman on the developing fetus.

There is ample evidence to suggest that the uterine environment plays an important role in the development of the fetus (Barr *et al.*, 1970). Alterations in this environment, such as the changes associated with maternal ingestion of alcohol or maternal smoking, have shown several deleterious effects on fetal outcome (Abel, 1982). Maternal alcohol consumption can depress the central nervous system

of the fetus and ingestion of large amounts of alcohol during pregnancy may lead to intrauterine growth retardation and congenital malformations; the fetal alcohol syndrome (Lewis and Boylan, 1979). Maternal smoking also has been associated with low birth weight infants, stillbirths and neonatal deaths, presumably by changing the uterine environment of the developing fetus (Socol *et al.*, 1982). It is also possible that maternal exercise may alter the fetal environment. For example, fetal breathing movements have been shown to increase during and after maternal activity of short duration (Marsal *et al.*, 1979). This increase may be the result of by-products such as lactic acid associated with maternal exercise and may ultimately affect fetal development.

One of the possible problems associated with maternal exercise is the shunting of maternal blood away from the uterus to the working muscles of the mother during normal reflex activity in response to exercise (Artal *et al.*, 1981). The possibility exists that lowered uteroplacental blood flow may result in diminished fetal oxygenation and fetal nutrition levels, at least for short periods of time as a result of the maternal exercise. At the termination of maternal activity, however, the uteroplacental blood flow is apparently restored in such a way that an overcompensation of available blood to the fetus occurs (Curet *et al.*, 1976). This phenomenon has been called the "tidal-flow" effect (Morris *et al.*, 1956; Martin, 1980). Whether in fact the

fetus is affected by this altered blood flow remains to be determined.

Decreased fetal oxygenation, or 'hypoxia' may also influence fetal outcome. The fetus reacts to hypoxic levels by the shunting of its own blood in favour of vital organs, such as heart and brain (Rudolf, 1984) until it can no longer cope, ultimately resulting in fetal death (Boddy, 1976). Less dramatic levels of fetal hypoxia have also been shown to cause growth retardation and smaller fetal organ weights (Longo *et al.*, 1978; Nelson *et al.*, 1983). However, during maternal exercise, diminished uterine blood flow does not necessarily indicate fetal hypoxia, as the fetus is somewhat protected by the mechanisms of the oxyhemoglobin dissociation curve and the strong affinity of fetal hemoglobin for oxygen (Longo, 1972). The level of diminished maternal-fetal blood flow which may lead to fetal hypoxia remains to be elucidated.

Fetal nutrition may also be affected by a decrease in maternal uteroplacental blood flow. Not only is the concentration of nutrients in maternal blood (an adequate maternal diet) essential for fetal growth, but an adequate maternal blood supply for transport of these nutrients to the fetus is also important (Young, 1976). For instance, the amount of glucose available to the fetus is necessary for fetal energy utilization (Bassett and Jones, 1976) and it is possible that maternal exercise may decrease the availability of glucose for the fetus. The maternal working

muscles may require glucose for energy especially at the more severe levels of maternal exercise rather than sparing glucose for fetal usage. Other essential nutrients such as protein and free fatty acids are also important for fetal growth. Skeletal muscle is especially susceptible to diminished levels of available protein (Young, 1976) and lipids are important in brain structure as well as being potential energy stores in later gestation (Hull, 1976). It appears then, that an adequate uteroplacental blood supply is essential in sustaining necessary fetal nutrition during pregnancy in order to maintain fetal growth and this supply may be threatened by maternal exercise.

Other aspects of maternal exercise which may affect fetal growth include alterations in circulating hormone levels associated with the combined effects of pregnancy and the intensities of maternal exercise. For instance, growth hormone levels have been found to increase during exercise (Cumming and Belcastro, 1982), but during pregnancy the levels of this hormone appear suppressed (Spellacy, 1977). Another example is the altered response of the adrenal medulla to exercise during pregnancy. Augmented circulating catecholamines associated with maternal exercise may affect fetal circulation (Cumming and Belcastro, 1982). The resultant addition of exercise to the altered hormonal environment of the pregnant woman and its effects on fetal outcome have not been fully elucidated.

Maternal hydration and blood volume levels may also be important in fetal growth. During pregnancy a significant amount of maternal body water is retained by the pregnant woman (Metcalfe *et al.*, 1981). Exercise on the other hand has been shown to decrease temporarily plasma volume (Hytten and Leitch, 1971). Whether maternal exercise causes changes in maternal hydration and plasma volume levels is not known. One of the possible consequences associated with maternal dehydration is oligohydramnios which has been linked with fetal abnormalities such as cleft palate or limb deformities, lung hypoplasia, and smaller birth weights (Symchych and Winchester, 1978). Whether maternal exercise causes diminished amounts of amniotic fluid is also undocumented.

Maternal exercise may also elevate maternal body temperature. Hyperthermia in the fetus has been shown to cause birth defects, especially in the fetal brain and intrauterine growth retardation (Edwards, 1974). It is not known, however, whether the extent of elevated maternal body temperature resulting from maternal exercise is sufficient to affect the developing fetus.

The picture surrounding the effects of maternal exercise seems far from complete and the illustrations outlined above show reasonable cause for concern. It is entirely possible that maternal exercise during gestation may help the development of the fetus in some circumstances whereas the same exercise may hinder the development of the

fetus in other circumstances. However, the level of maternal exercise and the degree of fitness considered to be "safe" or "recommended" during gestation have not been determined. There is increasing demand for such information as the influence of fitness and exercise during pregnancy becomes more prominent. While training may improve the capacity for maternal aerobic exercise, the effects of repeated maternal exercise on fetal well-being remain unclear (Cumming and Belcastro, 1982). Accordingly, this study was designed to examine the effects of three intensities of maternal aerobic exercise (mild, moderate and severe) on both mother and fetus throughout gestation in the rat.

The rat was chosen as the animal model rather than sheep, guinea-pigs or mice because the rat and human have the same type of haemochorial fetoplacental unit (Tuchmann-Duplessis *et al.*, 1972). Since the fetus is intimately related to the fetoplacental unit and the transfer and exchanges that occur between it and the maternal system, then effects of maternal exercise on the placenta or the substances that cross it would perhaps be reflected (in a similar manner to the human) in fetal rat growth and development.

Fetal growth and development were analysed in two ways:

1. The gross development of the fetus was represented by the following parameters:

   i. newborn number/litter and gross abnormalities

ii. newborn gross body weight

iii. newborn organ weights

2. At the microscopic level, skeletal muscle development was chosen for analysis. This fetal tissue was selected because it may reflect most readily any deficiency in fetal nutritional requirements which may be associated with maternal exercise.

The project was also designed to identify the effects of each of the three intensities of maternal exercise on the gross morphology of the maternal rat. The parameters selected to represent these effects were:

1. maternal weight gain during pregnancy
2. maternal postpartal weight
3. maternal skin (including subcutaneous fat and mammary glands) weight and the remaining body weight.

The study was conducted assuming the following limitations:

1. Sprague-Dawley rats were used throughout the experiment as a basis of an animal model for

maternal exercise during gestation. Rats are multiparous and the usual gestation period is 21 days. Pregnant rats are not susceptible to clinical complications due to an upright posture such as fluid retention or venous pooling in the legs and occlusion of the inferior vena cava by the gravid uterus (Metcalfe *et al.*, 1981).

2. The neonatal rats were sacrificed within 20 hours after birth which represented the fetal condition as a result of maternal exercise throughout gestation.

METHODOLOGY

The study consisted of three experiments conducted in succession over a period of three years. The effects of three intensities of maternal exercise were examined, commencing with a preliminary project of a mild aerobic exercise program (MILD). The mild experiment consisted of a treadmill speed of 15 metres/minute (m/min), on a 10° incline for a duration of 60 min/day, 5 days/week. This was based on a previous study by Parizkova (1975) who used a protocol of 15 m/min for 60 min/day. The results of the first (MILD) experiment served as a guide for the second which consisted of a moderate aerobic program (MOD) with a treadmill speed of 30 m/min (double the intensity of the first), on a 10° incline, for 60 min/day, 5 days/week. The third experiment, based on the results of the previous two, examined severe maternal aerobic exercise (SEV) which consisted of a treadmill speed of 30 m/min, on a 10° incline for a duration of 120 min (double the time of the second experiment). This program was based on a previous study by Bedford *et al.* (1979) who defined this intensity as 'heavy' aerobic exercise.

Several factors were common to all three experiments:

1. Young female Sprague-Dawley rats (Biosciences - University of Alberta) of approximately 50 days of age were used for study (N=20, MILD; N=30, MOD; N=30, SEV).

2. In the initial stages of each experiment, the female rats were weighed daily and randomly housed two per cage.

3. The ears were coded with an ear-hole punch to facilitate identification.

4. The rats were fed and provided with water *ad libitum*.

5. The animals were housed in the same room for each experiment. Daylight was controlled and included 12 hours of light (7:00 p.m.-7:00 a.m.) and 12 hours of darkness (7:00 a.m.-7:00 p.m.).

6. Room temperature was maintained at 72±2°F. Humidity was maintained between 45-55%.

PREPREGNANT PROGRESSIVE TREADMILL RUNNING PROGRAM:

The treadmill program for all running groups consisted of progressive treadmill exercise for two weeks. The rats were conditioned to run by a progressive running program so that at the end of the two weeks, they were capable of running the respective intensity (MILD, MOD or SEV) in the non-pregnant state. The protocol for progressive treadmill running was based on the progression schedules for rats used by Brooks and White (1978); Bedford *et al*. (1979); Davies *et al*. (1981); and Brooks and Donovan (1983). The MILD

progressive exercise program reached a final speed of 20 m/min, 10° incline, 60 min/day, 5 days/week (see Table 1). This exercise intensity has been reported elsewhere to elicit values less than 60% maximum oxygen comsumption ($\dot{V}O_2max$) for non-pregnant female Sprague-Dawley rats (Bedford *et al.*, 1979). The final exercise progression of the MOD experiment was 30 m/min, 10° incline, 60 min/day, 5 days/week (see Table 2). This intensity of exercise was found to elicit values of more than 60% $\dot{V}O_2max$ in non-pregnant female Sprague-Dawley rats (Bedford *et al.*, 1979). The SEV experiment reached a final progression of 30 m/min, 10° incline, 120 min/day, 5 days/week (see Table 3). Bedford *et al.* (1979) reported that in non-pregnant Sprague-Dawley rats this intensity of exercise elicited values greater than 80% $\dot{V}O_2max$.

Several factors were again common to each of the experiments:

1. The rats were exercised during the dark cycle, as rats are nocturnal animals and most physically active at night.

2. Each rat was weighed daily at approximately the same time just prior to running. An animal balance accurate to the nearest 0.1 g was used.

3. The treadmill was operated at the required speed for at least 20 minutes before the rats were placed on the machine. Even though the treadmill

Table 1. PREPREGNANT PROGRESSIVE TREADMILL RUNNING PROGRAM FOR THE MILD EXPERIMENT

DAY	TREADMILL SPEED (m/min)	INCLINE (°)	TIME (mins.)
1	15	10	15
2	15	10	20
3	15	10	30
4	15	10	40
5	20	10	50
6	REST		
7	REST		
8	20	10	60
9	20	10	60
10	20	10	60
11	20	10	60
12	20	10	60

Table 2. PREPREGNANT PROGRESSIVE TREADMILL RUNNING PROGRAM FOR THE MOD EXPERIMENT

DAY	TREADMILL SPEED (m/min)	INCLINE (°)	TIME (mins.)
1	15	10	15
2	15	10	20
3	20	10	30
4	20	10	40
5	25	10	50
6	REST		
7	REST		
8	25	10	60
9	26	10	60
10	28	10	60
11	30	10	60
12	30	10	60

Table 3. PREPREGNANT PROGRESSIVE TREADMILL RUNNING PROGRAM FOR THE SEV EXPERIMENT

DAY	TREADMILL SPEED (m/min)	INCLINE (°)	TIME (mins.)
1	20	10	15
2	23	10	20
3	25	10	30
4	28	10	45
5	30	10	60
6	REST		
7	REST		
8	30	10	60
9	30	10	75
10	30	10	90
11	30	10	105
12	30	10	120

was sufficiently warmed up, the speed of the belt altered slightly during the running sessions. On account of this, the belt was timed just before the rats began the exercise bouts and upon completion of exercise. The average of these two results was then recorded as the correct speed. The treadmill speed did not vary more than 1 m/min, however, for the duration of the running time.

4. A mild voltage (50 Volt) shock grid was located at the back of each treadmill runway.

5. A chart was kept of each rat's running behaviour to record injury or diarrhea (see Appendix A). If a rat was constantly shocked or injured while running, it was removed from the study.

6. At the end of the exercise routines the rat cages were always shifted to the right on a three tiered storage rack. The rats in the last cage always started the next exercise session so that a different animal began the running session each time.

7. At the end of the two week progressive running program (as soon as the exercise bout was complete) the rats were paired by weight.

GROUP DESCRIPTION:

These paired non-pregnant female rats were randomly selected by ear code identification to fulfill one of two main experimental conditions:

1. A pregnant group that continued their respective running intensity program throughout gestation (pregnant runner, PR-MILD; PR-MOD; PR-SEV), or

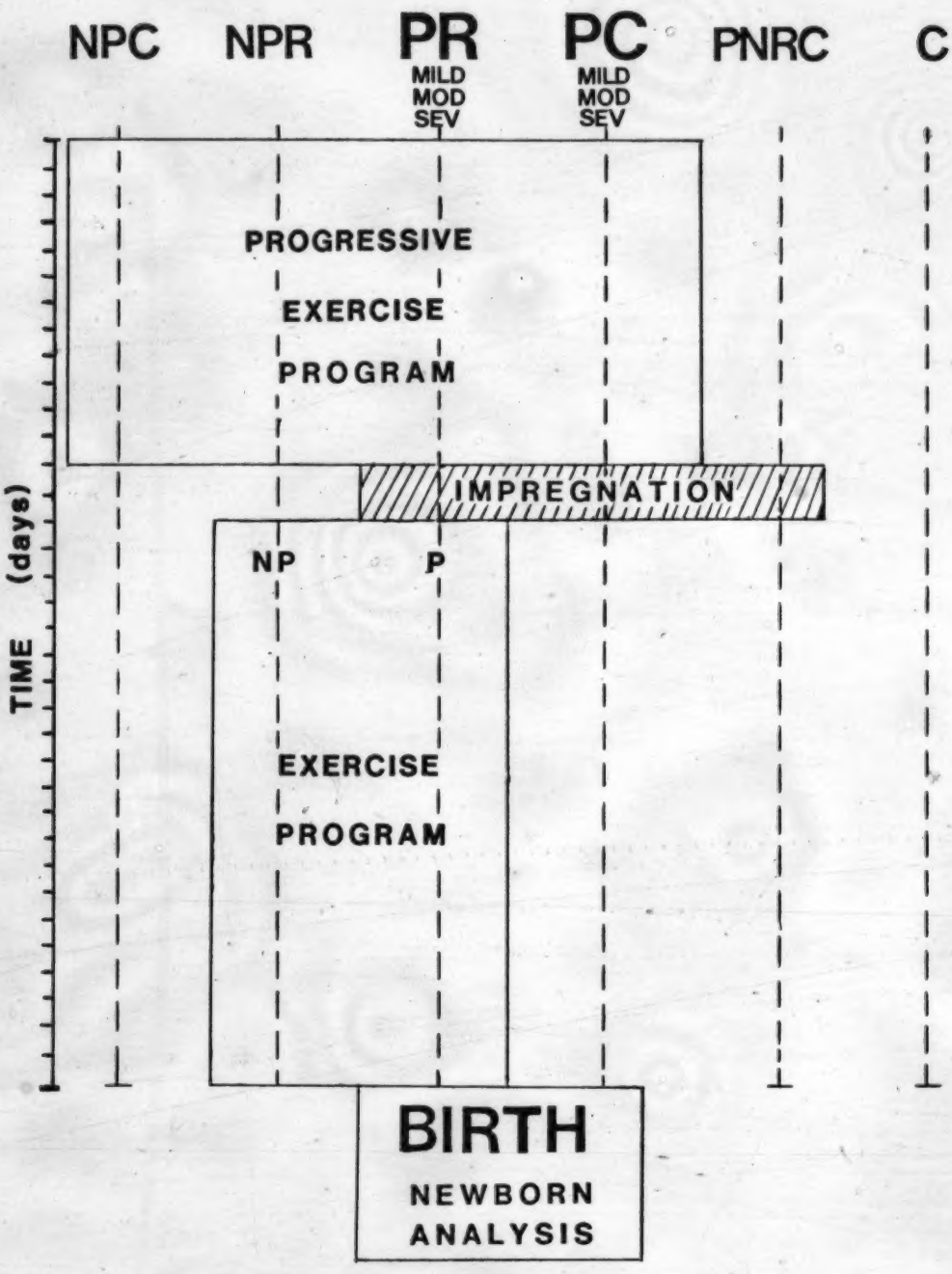
2. A pregnant group that did not continue the running program throughout pregnancy (pregnant control, PC-MILD; PC-MOD; PC-SEV).

In addition to the two main experimental groups, the following groups of rats were included in the general design (see figure 1) to observe the separate effects of exercise and/or pregnancy and to account for the normal weight gain of Sprague-Dawley rats:

1. a non-pregnant group that undertook the progressive treadmill running schedule, then stopped running and adopted a normal laboratory routine for the duration of the experiment (non-pregnant control, NPC)

2. a non-pregnant group that undertook the same progressive treadmill running schedule and then continued the running program (non-pregnant runner, NPR)

FIGURE 1. GENERAL DESIGN



3. a pregnant group that did not run and maintained normal laboratory routine for the duration of pregnancy (pregnant non-running control, PNRC)

4. a non-pregnant group that did not run and maintained normal laboratory routine for the duration of experimentation (control, C).

IMPREGNATION:

After identification of the appropriate groups, a male was introduced into the cages housing the paired female rats (PR and PC). The trio lived together until the females reached a weight of 300 g and exhibited definite abdominal swelling (this was based on the results from the MILD experiment). The females were then housed separately to eliminate mixing of the forthcoming litters. The separations occurred at approximately day 15, the beginning of the last third of pregnancy (gestation is about 21 days, Baker *et al.*, 1979).

RUNNING PROGRAM DURING PREGNANCY:

The PR-MILD group began the running program 4 days after the males were introduced into the cages. Four days of rest from exercise were given to increase the likelihood of pregnancy (estrous cycle = 4 days, Baker *et al.*, 1979). However, the results of the MILD experiment showed that this

was not necessary and consequently, PR-MOD and PR-SEV began the pregnant exercise program with only 2 days of rest. The PR rats continued their respective running program (MILD, MOD, or SEV) until the pups were born. The average treadmill speeds were again recorded as in the progressive treadmill exercise program by measuring the rate before and after each exercise bout. A record was also kept of each rat's running behaviour (see Appendix B) to determine how the pregnant rats were coping with the specific intensity of exercise. If a pregnant rat was consistently shocked or injured while running, it was removed from the study. However, if a superficial injury occurred, especially in the severe intensity experiment, an antiseptic was sprayed over the wound and the rat was usually given one day of rest from running. All the female rats were again weighed daily at approximately the same time, and on the days when exercise took place, just prior to the pregnant running rats' exercise session. The cages were again shifted to the right in the three tiered storage rack after completion of each exercise bout, so that the PR rat in the last cage ran first the following day.

In the SEV exercise experiment, because the running time for the PR group was 120 minutes, the PC group was removed from food, water and the influence of the male during the same time period. The additional two hours of food and water available to the PC group five times/week may have made a difference in body weight gain during pregnancy.

Using the outlined procedure, this variable was eliminated.

GROSS MORPHOLOGICAL ASSESSMENT OF NEONATES:

As soon after birth as possible, within a maximum of 20 hours (depending on when birth occurred), the number of newborns/litter and each pup's gross body weight was recorded. At this time the neonates were also checked for superficial gross abnormalities.

SEV Neonatal Postmortem Procedure:

Five newborn rats selected at random (using a random numbers table) from each litter in the SEV experiment only (PC and PR) were sacrificed using ether, as soon as possible (again within a maximum of 20 hours) after birth. The following organs were removed and immediately weighed: brain, heart, lungs, liver and kidneys. These organs were chosen for analysis to determine if the SEV exercise intensity altered fetal environment. Neonatal brain, heart, liver and kidney weight may reflect a reduction in fetal nutrition or fetal oxygenation. Neonatal lung weight may reflect alterations in amount of amniotic fluid.

The following procedure was used to remove the five organs:

1. The skin and skull were incised at the superior midline with a pair of scissors, postero-anteriorly. The entire brain was removed after severing the medulla oblongata at the

foramen magnum. The optic tissues were also included in the brain weights.

2. A midline incision was also made through the skin and sternum with a pair of scissors. The ribs were cut on the left side and the heart exposed. The heart was removed after cutting the great vessels at their roots.

3. Another incision was made on the right side of the thorax through skin and ribs to expose the right lung. Both lungs were removed after severing the bronchi and vessels at the hilus of the lung.

4. A midline incision was made in the abdomen. The liver was exposed in the right upper quadrant and removed after cutting any ligamentous material and vessels.

5. The kidneys were located retroperitoneally and were excised after cutting the structures at the renal hilus and also after detaching the adrenal glands.

All of the organs were weighed immediately after excision on a digital balance accurate to the nearest 0.001 g. The balance was cleaned after each weighing.

After excision of the specified organs in the same neonates of the PC-SEV and PR-SEV groups, three skeletal muscles were also removed; the right costal segment of the

diaphragm, the left gastrocnemius, and the medial left sternomastoid muscles. These three newborn skeletal muscles from different parts of the body were analysed to represent changes that may occur as a result of the maternal SEV exercise intensity. The diaphragm muscle was chosen for analysis because maternal exercise has been shown to increase fetal breathing movements (at least in the human) which are diaphragmatic in nature in the fetus and newborn (Manning, 1977). Perhaps any change in the development of this muscle due to increased usage would be reflected in the analysis. The sternomastoid and gastrocnemius muscles were chosen to represent the cephalic and caudal ends of the developing fetus, respectively. The sternomastoid muscle receives its blood supply from a branch of the external carotid and was selected to represent the cephalic end of the fetus. Gastrocnemius was chosen to represent a lower limb compartment from the caudal end. If fetal blood is redistributed from the caudal end of the fetus to favour the cranial end, this effect might also become manifest in the development of these particular muscles.

These neonatal muscle samples were placed in an agar block with the fibers oriented for a cross-sectional cut and fixed to a piece of cork, which was immediately frozen in isopentane cooled in liquid nitrogen (-160°C) (Bancroft and Stevens, 1982). The blocks were then stored at -60°C until sectioning.

Histological and Histochemical Procedures:

The neonatal skeletal muscle tissue was cut at -20°C in a cryostat at 12 μm thickness. The cut sections were placed on glass slides and stored at -60°C until staining. Just prior to staining the glass slides were dried at room temperature for 90 minutes. The slides were then stained using the following techniques:

1. Masson's trichrome stain (Dubowitz and Brooke, 1973) was used to colour nuclei, collagen and cytoplasm. This determined the histological morphology of the muscle tissue.

2. A modified Guth and Samaha (1969) myosin ATPase stain (preincubated at pH 10.4) procedure (see Appendix C) showed the ATPase enzymatic activity of the muscle fibers.

3. NADH-tetrazolium reductase (Dubowitz and Brooke, 1973) was used as a reaction for oxidative enzymes (which left a purple formazan deposit) and demonstrated the oxidative potentials of the muscle tissue (Bancroft and Stevens, 1982).

Stereological Assessment:

Black and white PAN-X film was used to photograph the skeletal muscle slides stained with Masson's trichrome from

the neonates in the SEV experiment (magnification X 40). The film was developed into negatives and a contact print was made of each roll of film. A Zeiss Jena plaque viewer projection unit was used to magnify the negatives (X 6.5). Stereological techniques were used to analyse the following muscle characteristics:

1. Muscle fiber diameter was analysed using the concentric circle test lattice technique outlined by Mayhew *et al*. (1977).

2. The nuclear position of the muscle fibers was observed and the percentage of centrally located nuclei (as opposed to peripheral) was determined.

3. The ratio of muscle tissue:connective tissue was assessed by using a line-grid test lattice (Weibel, 1973; Dias, 1974; Mayhew *et al*., 1977).

The grid area was 10 cm X 10 cm with the grid lattice lines 1 cm apart. The following formula was used to calculate the muscle tissue:connective tissue ratio.

$$Vv(a,c) = \frac{Pa}{Pc}$$

where:

$Vv(a,c)$ = volume density of component a (muscle) in the containing volume c.

Pa = number of test points falling on component a (muscle)

Pc = total number of test points (100).

With a test area of 10 cm X 10 cm the estimated error of Vv would be 10% (Weibel, 1979).

These stereological techniques were used to determine the histological development of neonatal skeletal muscles. These histolological assessments were based on Dubowitz (1968) who reported that rat newborn skeletal muscle had the following histological characteristics:

1. nuclei were relatively large and tended to be centrally located

2. muscle fibers were 'rounded' in shape (not polygonal as in adult muscle)

3. muscle fibers were grouped together loosely by endomysial connective tissue (not arranged in compact bundles as in adult muscle).

The neonatal diaphragm, sternomastoid and gastrocnemius muscles were analysed in this way to assess the effects of the SEV maternal exercise intensity on skeletal muscle development. The other slides stained with ATPase and NADH from the neonates in the SEV experiment were not photographed but were analysed microscopically in conjunction with serial sections treated with trichrome stain. These slides were assessed by direct observation to determine whether fiber differentation had occurred. If fiber differentiation had occurred, black and white PAN-X

film was again used to photograph the slides. The negatives were analysed using the same line-grid test lattice procedure to determine the ratio of type I muscle fibers.

GROSS MORPHOLOGICAL ASSESSMENT OF MATERNAL RATS:

The postpartal weights of all the maternal rats were recorded as soon after parturition as possible (within 20 hours, depending on when birth occurred). In addition to this the maternal rats in the SEV experiment were used to analyse maternal body components.

Postmortem Procedure:

The maternal rats in the SEV exercise experiment (PC and PR) were weighed postpartally and then sacrificed using ether. A midline ventral incision was made through the skin and subcutaneous tissue using sharp scissors. Care was taken not to disrupt the muscular tissue found deep to the subcutaneous tissue. The skin, subcutaneous fat and mammary tissue were then carefully dissected away from the muscle on the ventral aspect of the animal using a blunt close-open scissor technique. Care was taken not to damage the large vessels in the neck region. Some blood was lost when the vessels to the mammary glands were cut. A superficial incision was made around each limb immediately superior to the ankle joint. A longitudinal superficial cut through skin and subcutaneous tissue joined this incision to the ventral midline cut. Again, care was taken not to disturb any of the

underlying muscular tissue. Another circumferential superficial cut was made around the base of the tail and around the urogenital and rectal region. The rat was then placed in a prone position and the same blunt dissection technique was used to remove the skin and subcutaneous tissue. The subscapular fat pad was included with the skin and subcutaneous tissue. In the head region the ears were cut near the base and included with the skin portion. The skin was gently pulled over the head and removed from the body by a cut made at the end of the nose. The skin (which included subcutaneous tissue and mammary gland) was then weighed immediately on a digital balance to the nearest 0.1 g. The scale was cleaned after each weighing. The remainder of the carcass (including the tail) was also weighed to the nearest 0.1 g and both weights were recorded.

Another group of female rats, NPR, who underwent the same SEV exercise program as the PR group but who were not pregnant, were also sacrificed and skinned in the same manner at this time. The NPR skin weights and remaining carcass weights were also recorded to the nearest 0.1 g.

STATISTICAL ANALYSIS:

The data from the three experiments were examined individually using a paired Student's t-test (Avner, 1980) for each of the paired PC and PR groups (MILD, MOD and SEV). The PC and PR groups for MILD, MOD and SEV were then compared collectively using a one-way analysis of variance

(ANOVA) (Avner, 1980). Both of these statistical procedures were performed on each of the following:

1. the weights of the female rats on the day of conception,
2. the number of days prior to conception,
3. the last recorded pregnancy weights before giving birth,
4. the cumulative weight gained each day during pregnancy,
5. the postpartal weights for the pregnant groups within 20 hours after giving birth,
6. the postpartal weight gain (weight on day of conception minus postpartal weight),
7. the mean weights of each litter,
8. the average number of neonates per litter, and
9. the average total litter weights.

A three-way analysis of variance (ANOV30 - DERS, University of Alberta) using a repeated measures factor for time was performed on the weights of the female rats on day of conception, the last recorded pregnancy weights before giving birth and postpartal weights for the MILD, MOD and SEV experiments. In the SEV exercise experiment the maternal

skin weights and the carcass remainder weights of the PC and PR groups were compared to the NPR group using a one-way analysis of variance (Avner, 1980). Also in the SEV experiment, the newborn organ weights of the PR and PC groups were compared using a Student's t-test (Avner, 1980). The histological assessment of the neonatal skeletal muscle from the SEV experiment was statistically analysed using a one-way analysis of variance (Avner, 1980). Significance for all statistical tests was accepted at the $p<0.05$ level. Post-hoc analysis (Scheffé and Neumann-Keul Multiple Range test) was performed on significant ANOVA results.

RESULTS

The results will be presented in two sections with the neonatal data and maternal data separated to facilitate discussion. The neonatal data will be presented in four parts:

1. comparison of average values found for the neonates born to the PC and PR groups in each of the three exercise intensity experiments; MILD, MOD and SEV,

2. neonatal organ weight analysis from the SEV exercise experiment,

3. neonatal skeletal muscle analysis from the SEV exercise experiment,

4. summary of neonatal results.

The maternal data will also be presented in four parts;

1. maternal gross morphological data and maternal body weight gain during pregnancy (MILD, MOD, SEV),

2. maternal postpartal weight,

3. maternal body component analysis (skin weight and carcass remainder weight) for the SEV exercise experiment,

4. summary of maternal results.

NEONATAL DATA:

Table 4 shows average neonatal body weights, number per litter, total litter weights, abnormalities observed and mortality for the PC and PR groups for the MILD, MOD, and SEV experiments. No significant differences were found between any of the respective values for all of the three levels of exercise ($p>0.05$). In particular, the PR groups for each of the three exercise intensities gave birth to neonates with average weight values equivalent to their paired controls in the PC groups. None of the three exercise intensities studied caused a change in neonatal body weights. Further, the average number of neonates born per litter was not significantly different for the three exercise intensities ($p>0.05$). However, it is interesting to note that at all levels of exercise the PC groups gave birth to a larger average number of neonates per litter than the PR groups (approximately two in each case), even though these values were not significantly different. Mean total litter weight values of the two groups were also not significantly different, although the greater number of neonates found in the PC groups gave rise to the slightly higher total litter weight values.

Each neonate was also examined closely for gross superficial abnormalities, such as limb deformities and cleft palate. No abnormalities were observed. However, two neonates were found to be dead when the litters were examined. One was discovered in the PC-MILD group, the other

Table 4. NEONATAL DATA OF PREGNANT CONTROL AND PREGNANT RUNNING GROUPS FOR ALL THREE INTENSITIES OF MATERNAL EXERCISE*.

EXERCISE INTENSITY	MILD		MODERATE		SEVERE	
	PC	PR	PC	PR	PC	PR
WEIGHT (g)	7.0 (0.4)	7.3 (1.1)	6.7 (0.7)	6.7 (0.6)	6.8 (0.6)	6.9 (0.7)
#/LITTER	13.0 (1.6)	9.8 (2.9)	12.1 (1.2)	10.9 (3.2)	11.6 (2.5)	9.6 (1.7)
TOTAL LITTER WEIGHT (g)	90.7 (11.0)	70.4 (23.1)	80.8 (10.9)	71.2 (17.7)	78.2 (16.4)	65.9 (10.9)
ABNORMALITIES	none	none	none	none	none	none
MORTALITY	1	0	1	0	0	0

* - expressed as mean values (SD).

in the PC-MOD group. It was not known whether these neonates were stillborn or died after birth as post mortem examination was not performed. No neonatal deaths were found in any of the litters born to the PR groups.

Neonatal Organ Weights - SEV Experiment:

Table 5 presents the average wet organ weights for the five randomly selected neonates per litter in the PC-SEV (N=40) and PR-SEV (N=40) groups. No significant differences were found for any parameter. There were also no differences found in the ratios for neonatal brain weight:body weight, heart weight:body weight or in lung weight:body weight ($p>0.05$). The SEV exercise intensity did not cause a change in organ weights or in organ:body weight ratios in the neonates born to the PR group.

Neonatal Skeletal Muscle Analysis - SEV Experiment:

The results from the neonatal skeletal muscle analysis are found in Table 6 (see plates 1A and 1B; 2A and 2B in Appendix E). No significant differences were found between the PC and PR groups in the average muscle fiber diameters of gastrocnemius, sternomastoid or diaphragm muscles ($p>0.05$). However, the diaphragm muscle in both groups of neonates had significantly larger average fiber diameters than the average gastrocnemius and average sternomastoid fiber diameters ($p<0.05$).

Table 5. NEONATAL WET ORGAN WEIGHTS (g) AND RATIO VALUES FOR THE SEVERE EXERCISE GROUPS, PC & PR*

	PC	PR
NEONATAL BODY WEIGHTS	6.8 (0.600)	6.9 (0.700)
BRAIN WEIGHT	0.257 (0.030)	0.263 (0.027)
HEART WEIGHT	0.032 (0.005)	0.034 (0.005)
LIVER WEIGHT	0.344 (0.027)	0.337 (0.026)
KIDNEY WEIGHT	0.063 (0.010)	0.065 (0.011)
LUNG WEIGHT	0.116 (0.012)	0.114 (0.014)
BRAIN/BODY WEIGHT (%)	3.807 (0.265)	3.846 (0.214)
HEART/BODY WEIGHT (%)	0.478 (0.049)	0.489 (0.052)
LUNG/BODY WEIGHT (%)	1.720 (0.110)	1.690 (0.120)

* - expressed as mean values (SD).

Table 6. NEONATAL SKELETAL MUSCLE ANALYSIS FOR THE SEVERE EXERCISE GROUPS, PC & PR.**

	GASTROCNEMIUS		STERNOMASTOID		DIAPHRAGM	
	PC	PR	PC	PR	PC	PR
Fiber # Analysed	45.6 (9.3)	52.5 (11.9)	44.2 (4.4)	45.5 (5.7)	36.6 (3.2)	37.0 (4.4)
Fibers with central nuclei (%)	5.2 (6.1)	5.7 (7.2)	5.0 (3.7)	5.8 (5.2)	0.3 (0.9)	0.4 (1.1)
Muscle Fiber Diameter (mu)	3.1 (0.2)	3.2 (0.2)	3.7 (0.2)	3.8 (0.2)	4.5 (0.2)	4.5 (0.1)
Muscle:ct (%)	58.2 (10.2)	59.2 (8.5)	54.0 (7.5)	54.9 (8.6)	56.7 (5.7)	60.2 (7.0)
ATPase	ND	ND	ND	ND	*D	+D
NADH	ND	ND	ND	ND	ND	ND

** - expressed as mean values (SD).

c.t. - connective tissue

ND - Not Differentiated

D - Differentiated

* - Of total fiber # analysed/slide, 2.6 (0.6) % was type I

+ - Of total fiber # analysed/slide, 2.6 (0.7) % was type I

The average percentage of total muscle fibers analysed that contained centrally located nuclei is also presented in Table 6. Average values for the number of centrally placed nuclei in sternomastoid muscle were not different from gastrocnemius in either the PC or PR groups ($p>0.05$). It is interesting to note that the diaphragm muscle had significantly fewer muscle fibers with centrally placed nuclei than either the gastrocnemius or sternomastoid muscles in both the PC and PR groups ($p<0.05$).

Table 6 also demonstrates that the average ratio of muscle tissue:connective tissue was not significantly different in either group for all three muscles; gastrocnemius, sternomastoid, or diaphragm ($p>0.05$). In analysing the histological neonatal results, the SEV exercise intensity did not alter the development of sternomastoid, gastrocnemius or diaphragm muscles in the neonates of the PR group when compared to the neonates of the PC group. This exercise intensity also did not appear to alter the development of the caudal end represented by gastrocnemius muscle when compared to the cranial area represented by sternomastoid muscle in the PR neonates when compared to the control neonates of the PC group. Both cranial and caudal muscles from both groups of neonates did not differ histologically. However, the diaphragm muscle in both groups of neonates may be slightly more advanced developmentally because of the significantly fewer number of centrally placed nuclei.

The histochemical analysis of ATPase enzyme activity showed no neonatal muscle fiber differentiation of fiber types in either group for both gastrocnemius and sternomastoid muscles (see Table 6). The diaphragm muscle, however, did show differentiation into type I and type II fibers with regards to potential ATPase enzyme activity at a pH of 10.4. The average number of type I diaphragm muscle fibers counted per slide was not significantly different ($p>0.05$) for the neonates of the PC and PR groups. Both groups had the same percentage of type I muscle fibers in the diaphragm muscle. The histochemical reaction for NADH, however, failed to differentiate muscle fiber types in the diaphragm muscle, and also in sternomastoid and gastrocnemius muscles for either group of neonates. The SEV exercise intensity undertaken by the PR group did not appear to histochemically alter the neonatal skeletal muscles analysed when compared to the neonates of the PC group. The histochemical analysis of diaphragm muscle also indicated that this muscle may be more developed than both the gastrocnemius and sternomastoid muscles in both groups as type I and type II muscle fibers had differentiated in diaphragm muscle but not in the other two. However, diaphragm muscle enzymatic development was not complete because the NADH reaction failed to differentiate the muscle fibers into the appropriate fiber types.

Summary of Neonatal Results:

The results indicated no significant difference between the PC and PR groups for the MILD, MOD and SEV experiments with regard to average neonatal size, number per litter, or total litter weight. Furthermore, no superficial gross abnormalities were observed. Neonatal organ weights analysed from the SEV exercise experiment also exhibited no significant difference between the PC and PR groups, nor did the average heart:body weight, the brain:body weight and the lung:body weight ratios. Based on the histological and histochemical results, no difference was found between the neonates of the PC-SEV and the PR-SEV groups in skeletal muscle analysis. The diaphragm muscles of both groups of neonates (PC-SEV and PR-SEV) may have been more developed than the gastronemius and sternomastoid muscles of these same neonates because diaphragm muscle had fewer muscle fibers with centrally placed nuclei and the analysis of the enzymatic ATPase activity in diaphragm muscle showed differentiation into type I and type II muscle fibers. On the basis of the neonatal data presented, maternal exercise during pregnancy appeared to exhibit only minimal effects if any on the neonates.

MATERNAL DATA:

Gross Morphological Data and Body Weight Gain:

The gross morphological data for the PC and PR groups from each of the three exercise intensity experiments (MILD,

MOD, SEV) are found in Table 7. Of particular interest, is the fact that no significant differences were found between these groups in average weight on the day of conception ($p>0.05$). Also note from Table 7 that the three levels of exercise intensity did not appear to cause a significant delay in the number of days required for conception to occur after introduction of the male into the paired female's cages ($p>0.05$) (conception was estimated by counting back 21 days (gestation) from the day of birth).

It was also apparent that the MILD exercise intensity did not cause a significant reduction in the amount of average weight gained by the PR group compared to the PC group. The cumulative average weight gain values throughout gestation for both groups are plotted in figure 2 (the actual average values are found in Table 9 - Appendix D), as well as the average cumulative weight gained over this same time period by the non-pregnant normal C group for comparison. However, it is interesting to observe that the values for cumulative average weight gained by the PR-MILD group were consistently less than those found for the PC-MILD group throughout pregnancy ($p>0.05$), although the values were never sufficiently large to be significant. In contrast, the PC-MILD group on average had gained significantly more weight than the C group by day 9 of gestation and this difference continued until the end of pregnancy ($p<0.05$). This was also found for the PR-MILD

Table 7. GROSS MORPHOLOGICAL DATA FOR THE PREGNANT CONTROL, PREGNANT RUNNING GROUPS IN EACH EXERCISE INTENSITY - MILD, MODERATE, AND SEVERE**

GROUP ID	EXERCISE INTENSITY	WEIGHT (g) DAY OF CONCEPTION	CONCEPTION DELAY (days)	AMOUNT OF WT. GAINED DURING PREGNANCY (g)	POSTPARTAL WEIGHT GAIN (g)[1]
PC N=5	MILD	227.0 (20.2)	4.0 (4.5)	142.0 (14.0)	39.6 (10.1)
PR N=4	MILD	227.0 (20.6)	4.8 (2.6)	115.0 (17.0)	26.3 (15.1)
PC N=10	MOD	226.4 (10.5)	3.5 (2.5)	*144.3 (13.1)	'38.0 (9.0)
PR N=10	MOD	222.4 (17.3)	+7.0 (7.6)	*110.4 (22.9)	'27.2 (8.8)
PC N=8	SEV	239.8 (15.0)	6.5 (5.3)	++140.1 (18.9)	"43.6 (5.7)
PR N=8	SEV	231.4 (07.2)	6.4 (4.8)	++102.0 (10.2)	"30.4 (13.4)

** - expressed as mean values (SD).

\+ - one rat took 26 days to conceive

* - denotes significance; p=0.005 (ANOVA)

++ - denotes significance; p=0.004 (ANOVA)

' - denotes significance; p=0.0112 (Paired Student's t-test)

" - denotes significance; p=0.0419 (Paired Student's t-test)

[1] - value determined by average postpartal weight minus average weight on day of conception

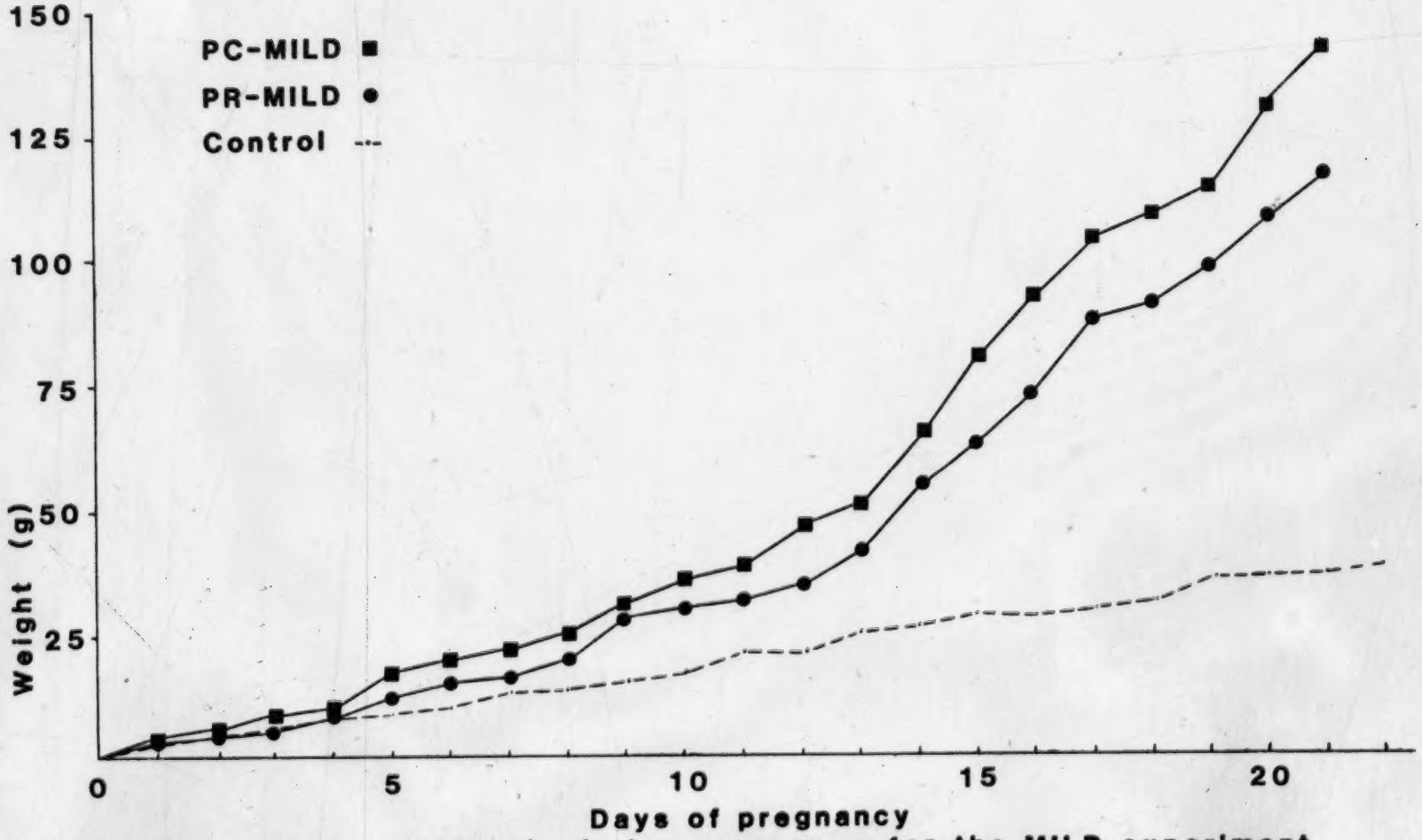


FIGURE 2. Average weight gain during pregnancy for the MILD experiment plotted against average weight gain of non-pregnant control group during same time period.

group although the significance in weight gain was delayed until day 14 of gestation. This significant difference also continued for the duration of pregnancy ($p<0.05$).

From Table 7 it can also be seen that the MOD and SEV exercise intensities appeared to cause a significant reduction in the average amount of weight gained by the maternal rats during pregnancy ($p<0.05$). The PC-MOD group gained an average of 33.9 g more than the PR-MOD group by the end of gestation and this difference became significantly large by day 8 of pregnancy ($p<0.05$) (see figure 3; actual average values found in Table 9 - Appendix D). The same values of cumulative average weight gain for the C group (as in figure 2) were also plotted in figure 3. Again, the PC-MOD group was significantly different from the C group in average weight gain (apparent by day 8 of gestation ($p<0.05$)). The PR-MOD group, however, did not become significantly different from the C group until day 16 of pregnancy.

From Table 7 it is also apparent that the PC-SEV group had gained significantly more weight than the PR-SEV group by day 11 of gestation and this difference was significant for the remainder of pregnancy ($p<0.05$) (see figure 4 and Table 9 - Appendix D). By the end of gestation, the PC-SEV group weighed 38.1 g more than the PR-SEV group ($p<0.05$). The average cumulative values for the C group in weight gained over this time period were also included in figure 4. Again, the PC-SEV group was found to be significantly

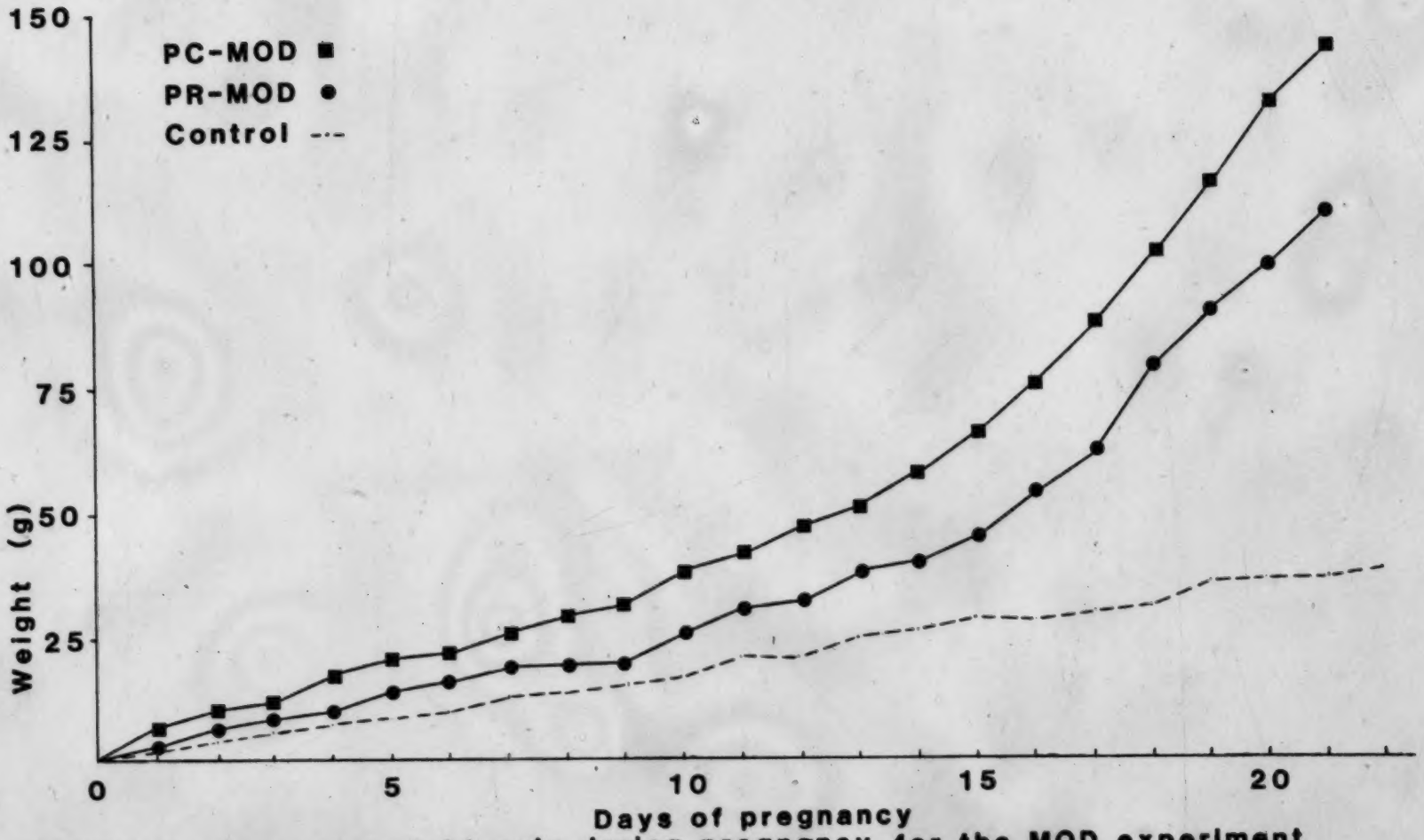


FIGURE 3. Average weight gain during pregnancy for the MOD experiment plotted against average weight gain of non-pregnant control group during same time period.

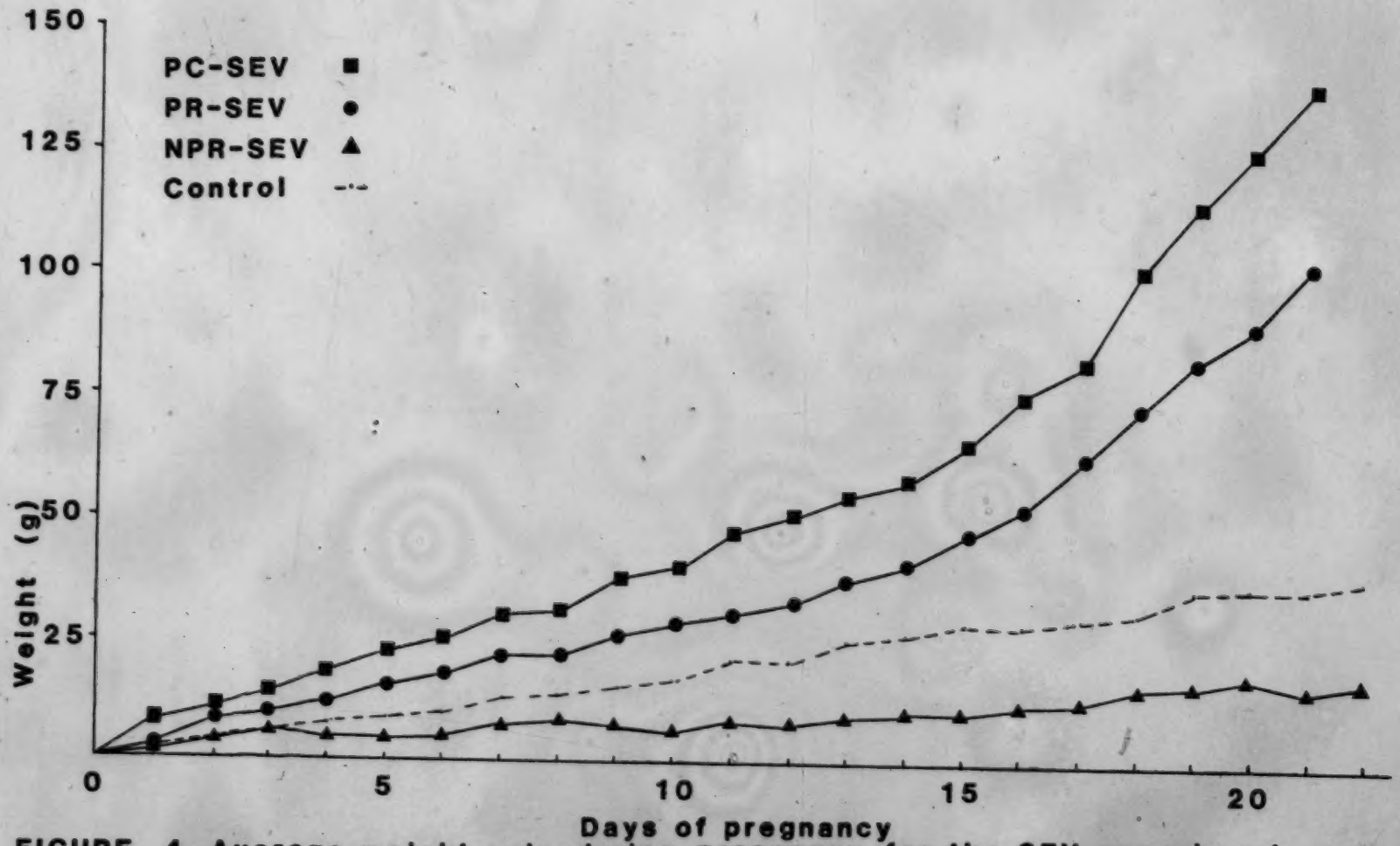


FIGURE 4. Average weight gain during pregnancy for the SEV experiment plotted against average weight gain of non-pregnant NPR and control groups during same time period.

different to the control group in body weight gain by day 6 of gestation and this difference was significant for the remainder of pregnancy ($p<0.05$). The PR-SEV group, however, did not become significantly different from the C group until day 16 of gestation (last third) and this difference also remained significant for the duration of pregnancy ($p<0.05$).

The cumulative values for average body weight gain from another group of non-pregnant rats, the NPR-SEV group, were also included in figure 4 (see also Table 9 - Appendix B). The amount of weight gained by the NPR-SEV group was found to be significantly less than the C group by day 11 of this time period. This significant difference continued between the two groups for the remainder of the experiment ($p<0.05$).

It is perhaps important to emphasise that both the MOD and SEV exercise intensities caused a reduction in maternal weight gain which became significant by day 8 (MOD) and day 11 (SEV) of gestation (mid-gestation). In both groups this difference remained significantly large for the rest of gestation. It is also important to note that both the PC-MOD and PC-SEV groups gained more weight than the non-pregnant C group and these differences became significant by days 8 and 6 of gestation, respectively (end of the first third). This is in contrast to both the PR groups (PR-MOD, PR-SEV) which also gained more weight than the non-pregnant C group but the increasing difference due to pregnancy appeared to be delayed and did not become significant until day 16 of

gestation (last third).

Maternal Postpartal Weight:

It is interesting to note that when the the maternal rats were weighed postpartally, the MILD exercise did not appear to influence postpartal weight gain values (postpartal weight minus weight on day of conception) when comparing the PR to the PC values ($p>0.05$) (see Table 7). However, when the MOD and SEV exercise intensities were examined, both the PR-MOD and PR-SEV groups had significantly lower postpartal weight gain values than their respective paired control group after having given birth ($p<0.05$) (see Table 7). It seems, therefore, that both the MOD and SEV exercise intensities appeared to affect maternal body weight gain throughout gestation and this effect remained manifest in the maternal weight gain values when examined postpartally.

When the overall effect of the three intensities of maternal exercise was examined on maternal postpartal weight gain values using the ANOVA method of analysis, no significant interaction effect was found between the three intensities of maternal exercise ($p>0.05$).

Maternal Body Component Analysis for the SEV Experiment:

Table 8 presents the results of the maternal body component analysis for the SEV exercise experiment. The body component analysis of the NPR group was also included in

Table 8. MATERNAL BODY COMPONENT ANALYSIS FOR THE SEV EXPERIMENT (expressed in g)*

GROUP	POSTPARTAL	CARCASS WEIGHT	
		SKIN WEIGHT	REMAINDER WEIGHT
PC N=7	282.7 (13.1)	53.6 (6.9)	227.5 (08.9)
PR N=7	257.6 (12.4)	43.1 (5.1)	211.8 (10.0)
NPR N=8	252.2 (11.5)	37.0 (3.7)	213.5 (08.2)

* - expressed as mean values (SD)

this table for comparison. Of particular interest is the fact that no significant difference was found between the average postpartal weight for the PR group (257.6 g) and the average weight on day 22 for the non-pregnant NPR group (252.2 g), even though the PR group had just given birth. In contrast, the PC group (282.7 g), which had also just given birth, had significantly heavier postpartal weights when compared to both the PR and the non-pregnant NPR groups ($p<0.05$).

It must be mentioned, however, that one rat from the PC group and another from the PR group were not included in the average weight values found in Table 8 due to severe hemorrhaging during the skinning process. These values may have incorrectly influenced the results. Also, for those rats included in the calculations, slight weight reduction occurred between pre- and post dissection total weight values due to small amounts of blood and fluid loss, but these losses were less than 1% of total body weight values.

The results of the maternal body component analysis indicated that the PC group had 10.5 g more of the skin component which included subcutaneous fat and mammary glands, than the PR group ($p<0.05$), whilst the average value for the remainder of the carcass was found to be 15.7 g more in the PC group when compared to the PR group ($p<0.05$). It is interesting to note that the PC group had a heavier average skin component and also a heavier carcass remainder component than the average PR values.

In comparing the results with the NPR group, it can be seen (Table 8) that the PR group had an average of 6.1 g more skin component, but this difference was not sufficiently large to be significant ($p>0.05$). The PC group, however, had an average value of 16.6 g more skin component than the non-pregnant NPR group and this was sufficiently large to be significant ($p<0.05$). The average weight found for the remainder of the carcass was almost identical in the PR (211.8 g) and NPR (213.5 g) groups, while the PC group (227.5 g) had an average value of 14.0 g more than the NPR group ($p<0.05$).

Summary of Maternal Results:

The results indicated that the three exercise intensities (MILD, MOD, and SEV) did not cause a delay in conception for the PR groups. Although the average body weight for the maternal rats did not differ on the day of conception in all groups, continuation of the MOD and SEV exercise intensities during pregnancy resulted in a significant reduction in body weight gain for the PR-MOD and PR-SEV groups by mid-gestation. This significant difference continued until parturition. The significant reduction in maternal body weight gain values was also found postpartum but again only for the PR-MOD and PR-SEV groups. In the SEV exercise experiment, the PC group had a heavier skin component and carcass remainder component than both the PR and the non-pregnant NPR groups, whilst the PR group did not

differ significantly from the NPR group in either of these body components. On the basis of the maternal data presented there appeared to be minimal effects on the maternal rats who undertook the MILD exercise intensity during pregnancy whilst the MOD and SEV exercise intensities caused a significant alteration in the maternal system as indicated by the reduction in maternal weight gain during gestation and after parturition.

DISCUSSION

Discrepancies exist in the literature with respect to the effects of maternal exercise on fetal growth and development. The factors which may contribute to these discrepancies are briefly mentioned here but are elucidated further in the Review of Literature (see page 104). In order to facilitate discussion of the present results with the current literature there are several factors that must first be considered.

There are three major factors upon reviewing the literature which may cause these discrepancies. One of these factors relates to the different animal species used (such as sheep, guinea-pigs, mice, rats or humans) for experimentation in maternal exercise. The exercise may affect each animal species in different ways and because of this the following discussion will be centred mainly on maternal mice, rats and guinea pigs, with emphasis on studies pertaining to rat data. The second major factor concerns the familiarization and/or training of the maternal animal before pregnancy. If the animal is not familiarized to the equipment before experimentation is initiated, the stress of a different environment may compound the actual stress of the exercise on the pregnant animal. Also the training itself leads to many physiological alterations in the non-pregnant animal (Cumming and Belcastro, 1982; Cumming and Rebar, 1983) which may alter the animal's response to exercise during pregnancy and the resultant

fetal outcome. The third major factor is the use of different exercise intensities which includes varying the duration of the exercise, speed of the treadmill, and inclination of the treadmill. It is difficult to compare the effects of maternal exercise between different studies when the intensities of exercise are different. In human studies another variable, that of weight bearing exercise (treadmill running) as opposed to non-weight bearing exercise (bicycle ergometer) is added to compound the problem. The extra weight of pregnancy in addition to the maternal exercise may stress the pregnant woman differently than if her weight was supported and this may also alter the results.

Several other factors, also leading to discrepancies in the literature, should not go without mention. For instance, the technique used to "prompt" the animal to run, such as electric shock grids may add undue stress on the exercising animal, affecting the results (Wilson and Gisolfi, 1980). Another consideration is whether invasive techniques are used to monitor maternal heart rate or fetal blood flow, while the maternal animals are exercising, for this may also place stress on the maternal and fetal systems. Depriving the maternal animal of food or water for any length of time before the maternal exercise, may also alter the results because nutrition is an important element in fetal growth and development (Young, 1976). All of these different factors must be considered as they may affect the results and make difficult any comparisons with the available

literature. The discussion in the present study will be presented in two major sections: neonatal data and maternal data.

NEONATAL DATA:

The results of the present study showed that average neonatal body weights from all three maternal exercise intensities (MILD, MOD, and SEV) were not significantly different from the control values. This agreed with the findings of Parizkova, 1975; Erkkola, 1976b; Dressendorfer, 1978; Parizkova and Petrasek, 1978; Gilbert *et al.*, 1979; Collings *et al.*, 1981; Harrison, 1981; Ruhling *et al.*, 1981; Woodward, 1981; and Collings *et al.*, 1983, all of whom did not demonstrate a reduction in fetal or neonatal body weights as a result of maternal exercise.

Analysis of newborns and fetal outcome measurements are important in assessing life *in utero*. Neonatal body weight values are a good indicator of a completely functioning intrauterine environment (Rush, 1973). If the intrauterine environment becomes compromised in some way, causing intrauterine insufficiency to occur during prenatal life, fetal growth retardation is characterized by a significant reduction in neonatal body weight at birth (Vorherr, 1982). One major factor contributing to intrauterine growth retardation is a marked reduction in materno-placental blood flow (Gilbert and Leturque, 1982) such as that which may be associated with maternal exercise, attributable to the

shunting of uteroplacental blood to the working muscles of the mother during exercise (Morris *et al.*, 1956; Longo *et al.*, 1978; Pernoll *et al.*, 1978; Artal *et al.*, 1981). In support of this, several authors have shown a reduction in fetal body weight values as a result of maternal exercise (Terada, 1974; Dhindsa *et al.*, 1978; Longo *et al.*, 1978; Gilbert *et al.*, 1981; Nelson *et al.*, 1983), which would disagree with the results of the present study. The reduction in uteroplacental blood flow may result in intrauterine growth retardation because of the decrease in oxygen availability and reduced levels of nutrition available to the developing fetus (Young, 1976). However, there are other groups of researchers who would dispute the alterations in uteroplacental blood flow and fetal outcome associated with maternal exercise. For example, Curet *et al.* (1976) and Dale *et al.* (1982) reported no significant reduction in uteroplacental blood flow concomitant with maternal exercise.

The average neonatal body weights found in the present study did not differ from average control neonatal weights for rats presented in other reports (Buhrdel *et al.*, 1978; Palou *et al.*, 1983). However, Buhrdel *et al.* (1978) and Palou *et al.* (1983) used Wistar rats in their reports on neonatal development and this must be borne in mind when comparisons are made to the present study in which Sprague-Dawley rats were used for experimentation.

When the literature was reviewed for control values of the litter sizes for rats, a range of 8 to 16 offspring per litter were found. The average value for the litter size of control rats in the study by Buhrdel *et al*. (1978) using Wistar rats was reported as 8.85 ±2.56. This average value was within the control range of approximately 9 to 12 offspring per litter found in the experiments of Parizkova (1975); and Parizkova and Petrasek (1978), who also used Wistar rats. Wilson and Gisolfi (1980) found an average value of 9.5 ±3.5 neonates per litter in control Sprague-Dawley rats. Atherton *et al*. (1982) reported an average range of 8 to 16 offspring also born to control Sprague-Dawley rats. The average litter values in the present study were found to be within these normal ranges reported for both Wistar and Sprague-Dawley rats. It would appear that maternal exercise in the present study did not cause a change in the average number of neonates found per litter in maternal rats who ran during pregnancy. The results of the present study confirm reports by Parizkova, 1975; Parizkova and Petrasek, 1978; and Wilson and Gisolfi, 1980; all of whom reported no change in average litter size as a result of maternal exercise in rats.

With regards to total litter weight values, Vorherr (1982) reported that in rats, the total birth weight of a litter should be approximately 20% that of maternal weight. With a mean litter size of 10 offspring, the birth weight of one pup would be 2% that of the maternal rat (Vorherr,

1982). When these proportions were examined in the present study it was found that in all the maternal groups of rats, the average litter weight values were not significantly different from 20% of maternal weight and the proportionate percentage of one offspring (average neonatal weight) to average maternal weight was not significantly different from 2% (see Table 10 in Appendix D). These average values again confirm the concept that no differences in fetal outcome were apparent when the offspring of exercised mothers were compared to those from control animals.

Another factor which may contribute to intrauterine growth retardation and the reported smaller birth weight values found in the literature, is a decrease in the amount of amniotic fluid (oligohydramnios). In rats, oligohydramnios has been associated with smaller birth weights, fetal abnormalities such as cleft palate, limb deformities and an increase in fetal mortality (Symchych and Winchester, 1978). It is not presently known whether the combination of exercise and pregnancy may lead to maternal dehydration, but a diminished maternal blood volume may consequently reduce the amount of amniotic fluid leading to oligohydramnios (Longo, 1983). A few studies have reported an increase in mortality rates and smaller birth weights in the offspring of mothers who ran during pregnancy (Terada, 1974; Wilson and Gisolfi, 1980), but these results would disagree with the findings of the present study. The association of oligohydramnios, intrauterine growth

retardation and maternal exercise has not yet been investigated.

One possible link between maternal exercise and intrauterine growth retardation centres on the fact that maternal exercise may also elevate maternal body temperature (Medical Tribune World Service, 1983). Exercise in non-pregnant rats has been shown to elevate core body temperature (Francesconi and Mayer, 1983). Hyperthermia in the rat fetus has been shown to cause birth defects, especially in the fetal brain and intrauterine growth retardation (Edwards, 1974). It is not known, however, whether the extent of assumed elevated maternal body temperature resulting from maternal exercise is sufficient to affect the developing fetus. Each neonate in the present study was examined for superficial gross abnormalities but none were observed; nor were the birth weight values significantly reduced in the neonates of the maternal rats who ran during pregnancy. This is in contrast to Terada (1974) who reported that pregnant mice (untrained) forced to run repetitively during mid-gestation exhibited an increase in fetal mortality (including resorption and maceration), a decrease in fetal body weight and a delay in fetal skeletal ossification when compared to control values. It is not known, however, whether oligohydramnios, maternal hyperthermia or a diminished uteroplacental blood flow, or any, or all of these factors, were contributors to alterations in fetal outcome found in Terada's (1974) study.

Terada (1974) also reported that in another group of mice who were trained before pregnancy, no fetal malformations were observed and fetal mortality rates were reduced when compared to the results of his previous group of mice who ran only during mid-gestation. This is important to note because the results of Terada's (1974) study would suggest that training before pregnancy may have reduced the otherwise teratologic effect of maternal exercise in fetal mice. In the present study the maternal rats were subjected to a prepregnancy progressive running program which corresponded to the specific exercise intensity to be run during pregnancy by the MILD, MOD and SEV groups, respectively. The progressive running programs were not specifically designed to "train" the non-pregnant rats, but rather to accustom them to treadmill running. Physiological alterations associated with training, such as increased cytochrome oxidase activity in skeletal leg muscle, or in $\dot{V}O_2max$, for example, were not examined in the present study. The two week prepregnancy program of running, however, may have played a role in reducing the effects of maternal exercise on the fetus. These results would support the theory exemplified in Terada's (1974) study and also shown in other studies (Curet *et al.*, 1976 (sheep, 3 weeks prior training); Dressendorfer, 1978 (human, several months prior training); Wilson and Gisolfi, 1980 (rat, 7 weeks prior training)), that prepregnancy training and familiarization may somehow protect the developing fetus from otherwise

harmful effects during maternal exercise. This theory is also supported by the fact that many researchers who did find reductions in uteroplacental blood flow and smaller fetal body weights linked to maternal exercise, did not report training or familiarizing of their maternal animals to running before pregnancy (Morris *et al.*, 1956 (human); Longo *et al.*, 1978 (sheep); Gilbert *et al.*, 1981 (guinea-pigs); Nelson *et al.*, 1983 (guinea-pigs)).

It would seem then that perhaps acute repetitive maternal exercise during mid-gestation may affect the fetal environment. The mechanisms involved which may alter fetal environment are not known but perhaps a diminished uteroplacental blood supply may be one of the key issues.

In accordance with this, two interesting studies by Bruce (1976; 1977), in which he ligated one uterine artery in a pregnant rat at various times during gestation, produced important results. He found that ligating a uterine artery on day 17 of gestation in the rat caused fetal death and growth retardation from an impaired uteroplacental circulation. However, ligation on days 1, 2 or 7 of gestation had no affect on fetal development and on day 10 caused only a small decrease in fetal weights (Bruce, 1976). In early gestation, this lack of effect on fetal growth was attributed to the anastomotic development of the ovarian and hypogastric arteries (Bruce, 1977). This adaptive mechanism in early pregnancy appeared to restore the diminished uteroplacental blood supply to the necessary level for

normal fetal development. In the later stages of gestation, however, this mechanism could not restore uteroplacental blood to the appropriate level and fetal death occurred. The results of Bruce's (1976; 1977) studies may support the concept that perhaps a diminished uteroplacental blood supply, such as that which may occur as a result of acute repetitive maternal exercise in mid-gestation, may alter the fetal environment sufficiently to cause fetal growth retardation and an increase in fetal mortality as seen in Terada's (1974) study on pregnant mice. Adaptive mechanisms that are normally present during gestation such as the oxyhemoglobin dissociation curve and the strong affinity of fetal hemoglobin for oxygen (Longo, 1972), may not be sufficient to cope with the stress of acute maternal exercise and therefore fetal development is affected. In applying Bruce's (1977) adaptation principle, maternal exercise prior to pregnancy, or exercise begun in the early stages of pregnancy may enable adaptational mechanisms to develop as maternal exercise is continued throughout gestation. It is possible that training and familiarization before pregnancy or during early pregnancy may reduce the changes associated with maternal exercise on the fetal environment, perhaps allowing the fetus to cope more effectively.

One of the ways in which training may reduce the alterations in the fetal enviromnent is by preserving uteroplacental blood flow during exercise. The splanchnic

blood flow measured in trained animals was maintained during hard exercise, while in untrained animals this blood reserve was shunted toward the exercising skeletal muscles (Donovan and Brooks, 1983). In accordance with this, Curet *et al.* (1976) observed that at the completion of maternal exercise, uteroplacental blood flow was apparently restored in such a way that overcompensation of available blood to the fetus occurred. Perhaps this "tidal-flow" effect (Morris *et al*, 1956; Martin, 1980), in combination with maintenance of splanchnic blood flow that occurs with training, may serve to protect the fetus in trained animals from a diminished uteroplacental blood supply. Further testing of this important hypothesis is necessary.

Neonatal Organ Weights:

One of the problems linked with a diminished uteroplacental blood supply, such as that associated with acute repetitive maternal exercise, is that the fetus may also have reduced oxygen availability (Emmanouilides, 1972; Longo *et al.*, 1978). This association was suggested from the observation that in cannulated sheep fetuses there was as much as a 59% reduction in pO_2 values during acute maternal exercise (Longo *et al.*, 1978). Reduced fetal oxygen availability or 'hypoxia', has been linked to fetal growth retardation and smaller fetal organ weight values and in extreme cases of hypoxia, the fetus reacts by the shunting of its own blood to favour vital organs, such as the heart

and brain (Boddy, 1976; Rudolf, 1984). This latter reaction would become manifest by larger ratios of brain weight:fetal body weight and heart weight:fetal body weight because of the smaller fetal body size and the disproportionately larger brain and heart resulting from the redistribution of fetal blood to favour these organs (Gilbert *et al.*, 1979). In the present study neonatal organ weight values and the ratio of these values to neonatal body weight were examined, but only in the SEV experiment where the most significant changes may have occurred. No significant differences were found between the organ weight values of the neonates born to the PC and PR groups. The neonatal organ weight values found in the present study were also not significantly different from newborn control values of Wistar rats reported by Palou *et al.* (1983) (average kidney weight 0.064±0.002 g; average brain weight 0.255±0.008 g; average liver weight 0.251±0.015 g; see Table 5 for comparison). There was also no difference found in the heart:body weight and brain:body weight ratios in the neonates of both groups. This would suggest that perhaps even maternal exercise of the SEV intensity experiment did not reduce oxygen availability to the fetus or that, perhaps so little oxygen reduction occurred that the fetus adapted well to its changing environment. This may be due to adaptational mechanisms that may develop along with the fetus throughout gestation in trained maternal rats.

A major concern associated with diminished uteroplacental blood flow, also, is the reduction in fetal nutrition levels as a result of maternal exercise. An adequate amount of glucose available to the fetus is necessary for fetal energy utilization (Bassett and Jones, 1976), and it is possible that maternal exercise may decrease the availability of glucose for the fetus by requiring glucose for energy in the maternal working muscles, especially at the more severe levels of maternal exercise. A diminished level of energy substrate may lead to fetoplacental growth retardation. A reduced level in lipid availablity may also lead to smaller fetal body weight values because lipids are important potential energy stores especially in the later stages of gestation (Hull, 1976). Lipids are also required for the developing brain structure (Hull, 1976) and consequently a diminished supply may be reflected in smaller fetal brain weights. In the present study, no reduction was found in neonatal brain weights or in the body weights of offspring born to the PR-SEV group. This may indicate that fetal nutrition levels were not altered or sufficiently reduced to elicit changes in fetal growth or, again, adaptational mechanisms had developed from the beginning of gestation.

In contrast to the minimal effects of the SEV exercise intensity on neonatal organ weight values found in the present study, one group of authors have reported alterations in fetal guinea-pig body and organ weights with

acute repetitive maternal exercise (no prepregnancy training) (Gilbert *et al.*, 1981; Nelson *et al.*, 1983). Gilbert *et al.* (1981) reported that guinea-pig fetal body weight, placental weight and fetal kidney weight exhibited a reduction in average weight with increasing levels of maternal exercise. Fetal heart and brain weights did not exhibit a change with increasing maternal exercise level. However, heart weight:fetal body weight and brain weight:fetal body weight ratios increased at the higher levels of maternal exercise (Gilbert *et al.*, 1981). Nelson *et al.* (1983) also reported that in the fetuses of exercising pregnant guinea-pigs, fetal body weight, kidney weight and placenta weight were reduced as the level of maternal exercise increased. However, ratios of heart, kidney and placental weights:fetal body weight decreased significantly from the control values, but at the highest maternal exercise intensity level (60 min/day), the brain weight:fetal body weight ratio was significantly increased from control values (Nelson *et al.*, 1983).

Gilbert *et al.* (1979), however, in a previous study found no differences in guinea-pig fetal body weights, organ weights or in placental weights, as a result of acute repetitive maternal exercise and no difference in the ratio of fetal organ weights:body weights. Gilbert *et al.* (1981) and Nelson *et al.* (1983) attributed this contrasting result in their research group to a lower level of maternal exercise. The pregnant guinea-pigs in the study by Gilbert

et al. (1979) ran at a less intense level of exercise than the pregnant guinea-pigs of Gilbert *et al.* (1981) and Nelson *et al.*, (1983). From the results presented in their laboratory, increasing levels of acute maternal exercise seemed to cause more alterations in the fetal environment as manifest by the changes in fetal organ weights and reduced fetal body weights. It would seem then that the intensity of acute repetitive maternal exercise is also an important factor to consider.

In the present study three maternal exercise intensities (MILD, MOD and SEV) were examined throughout pregnancy and no differences were found in any of these groups with regard to neonatal body weight values. Even in the most SEV exercise intensity, no differences were found in neonatal organ weight values. It would appear that prepregnancy exercise and exercise in early gestation may again promote adaptive mechanisms to develop which may protect the fetus from the effects of even the more intense maternal exercise. The results of Wilson and Gisolfi (1980) lend support to the results of the present study in that they did not find significant differences in rat offspring body dimensions (body and tail lengths) or organ weights (heart, spleen, kidney and adrenal) between the trained (7 week prepregnancy training program at an intensity similar to the SEV experiment in the present study) pregnant rats who also ran throughout pregnancy and their control group. In comparison, <u>acute</u> repetitive maternal exercise at the

higher intensity levels may perhaps "breakdown" adaptational mechanisms which still may exist at the mild intensity level used by Gilbert *et al*. (1979). It would seem likely that acute repetitive maternal exercise and intensity of that exercise are important factors, both of which may alter fetal responses. It is difficult, however, to equate the exercise intensity level used for pregnant guinea-pigs to the level used for pregnant rats.

One other factor that should also be considered is the length of gestation in pregnant guinea-pigs which is approximately 67 days (Nelson *et al*., 1983), while in rats, gestation terminates at about 21 days (Baker *et al*., 1979). Rats are also more immature at birth than are newborn guinea-pigs (Dubowitz, 1968) and it may be possible that the acute affects of maternal exercise coupled with a longer time spent *in utero* may manifest fetal alterations in the guinea-pig which may not have occurred in the more immature rat fetus. Nevertheless, it may be increasingly apparent that training throughout pregnancy may perhaps promote adaptive mechanisms which may protect the fetus, even at levels of severe maternal exercise, while the stress of acute repetitive maternal exercise beyond a certain intensity may elicit alterations in fetal environment.

There was also one more organ weight value examined in the present study which may support the adaptive mechanism theory. The ratio of average lung weight:body weight was calculated for the neonates born to the PC-SEV and PR-SEV

groups and these values were not significantly different. Symchych and Winchester (1978) stated that adequate amounts of amniotic fluid were an essential component for normal lung growth and experimental deficiency of amniotic fluid (such as that associated with oligohydramnios) in the last third of gestation may be linked with pulmonary hypoplasia and significant decreases in average lung:body weight ratios. The fact that lung weight and lung weight:body weight ratios were not different in the neonates of the PR group when compared to the controls, would perhaps lend additional support to the presence of adequate amounts of amniotic fluid so that symptoms associated with oligohydramnios were not manifested. This may also support the theory that adaptive mechanisms were perhaps present that not only protected the fetus from the effects of a diminished uteroplacental blood supply, but also from alterations in the amount of amniotic fluid.

It would appear that the alterations in fetal outcome associated with maternal exercise reported in the literature deal with the higher intensities of <u>acute</u> repetitive maternal exercise during mid-gestation as the milder intensities of acute maternal exercise did not appear to exhibit these fetal alterations. It may be possible that the fetus is capable of coping with a certain level of alteration in its environment beyond which problems in fetal growth become manifested. Training and familiarization before pregnancy and during early pregnancy may somehow

reduce the teratogenic effects of acute maternal exercise on the fetus by the development of adaptive mechanisms. These mechanisms have yet to be elucidated, but in the rat, anastomotic channels have been found after ligation of a uterine artery in early pregnancy (Bruce, 1976, 1977). The "tidal-effect" reported after maternal exercise (Morris *et al.*, 1956; Martin, 1980) may also act as an adaptive mechanism perhaps found in the "surplus" of available blood. These adaptive mechanisms appear to protect the fetus in a trained and familiarized group of animals even at the more severe maternal exercise intensities. The gross morphological neonatal results reported in the present study would support this theory. However, if alterations exist in the neonates of the most severe exercise intensity group in the present study, changes may not be manifested at the gross morphological level but rather more subtly at perhaps the histological or histochemical level.

Neonatal Skeletal Muscle:

Developing fetal skeletal muscle is especially susceptible to diminished levels of available maternal protein, which may occur as a result of a diminished uteroplacental blood flow (Young, 1976). Neonatal skeletal muscle was analysed in the present study to determine if skeletal muscle tissue was altered histologically or histochemically in the neonates of the PR-SEV group when compared to the neonates born to the PC-SEV group.

It has been reported that neonatal skeletal muscle in the rat is relatively undifferentiated and uniformly stains positive for enzyme reactions at birth (Dubowitz, 1965; Dubowitz, 1968; Engel and Karpati, 1968; Ashmore *et al.*, 1972; Shafiq *et al.*, 1972; Drachman and Johnson, 1973; Haltia *et al.*, 1978; Villa-Moruzzi *et al.*, 1979; Ho *et al.*, 1983). This was confirmed by the results of the present study. Intense actomyosin ATPase activity has been demonstrated in all hindlimb muscle fibers of the newborn rat (Dubowitz, 1968; Ashmore *et al.*, 1972) and these muscle fibers were also found to stain positively for NADH reactions (Dubowitz, 1968). Dubowitz (1968) also showed that in a cross-section of newborn hindlimb rat muscle stained with haematoxalin and eosin, the muscle fibers were rounded (not polygonal as in adult muscle), and grouped together loosely (not arranged in compact bundles as in adult muscle) by endomysial connective tissue. The nuclei were relatively large and tended to be centrally located (Dubowitz, 1968). This descriptive analysis is indicative of newborn skeletal muscle.

In the present study, the histological appearance of gastrocnemius and sternomastoid muscles would agree with that described by Dubowitz (1968) for normal rat muscle. The trichrome stain (used to show histological features in the present study) also showed rounded muscle fibers with both gastrocnemius and sternomastoid muscle fibers containing some centrally placed nuclei. The ratios of muscle

tissue:connective tissue found for both gastrocnemius and sternomastoid muscles also indicated there was a relatively large amount of connective tissue present. The histochemical analysis of gastrocnemius and sternomastoid muscles in the present study also agreed with the description given by Dubowitz (1968); Engel and Karpati (1968); Ashmore *et al.* (1972); Shafiq *et al.* (1972); Haltia *et al.* (1978); Ho *et al.* (1983), for normal rat neonatal skeletal muscles, with respect to uniform enzyme staining for myosin ATPase and NADH histochemical procedures which were unable to differentiate muscle fiber types. The neonatal gastrocnemius and sternomastoid muscles for the PC-SEV and PR-SEV groups were not found to be significantly different in the histological or histochemical parameters measured and both groups exhibited typical newborn rat skeletal muscle characteristics.

A similar analysis of diaphragm muscle in the present study for both PC-SEV and PR-SEV groups showed significantly larger fiber diameters and significantly fewer muscle fibers with centrally placed nuclei than either gastrocnemius or sternomastoid muscles. When diaphragm muscle was analysed histochemically, myosin ATPase enzyme activity was found to differentiate the muscle fibers into type I and type II fibers. Diaphragm type I fibers were found to occupy only 2.6 (0.6)% of the total fiber number counted per slide in both the PC-SEV and PR-SEV groups. The histochemical reaction for NADH in the diaphragm muscle for both groups of

neonates failed to show differentiation into the appropriate fiber types. Dubowitz (1968) reported, however, that the NADH reaction into fiber types does not usually become evident until approximately day 14 postnatally in the rat. Again no differences were found between the neonates of the PC-SEV and PR-SEV groups in any of the histological or histochemical parameters measured for diaphragm muscle.

It would appear that neonatal rat skeletal muscle usually has not differentiated into fiber types based on enzymatic activity of the muscle fibers at birth as this process of differentiation does not normally occur until 14 days postnatally (Dubowitz, 1968). The diaphragm, however, seemed to be more developed than most neonatal skeletal muscle because of the smaller numbers of centrally placed nuclei, and histochemically the diaphragm muscle tissue had begun to show enzymatic fiber types. It would seem logical, however, for diaphragm muscle to be slightly more advanced developmentally than either sternomastoid or gastrocnemius because of the functional importance of the diaphragm in breathing in the newborn. Breathing movements in the fetus and breathing movements in the newborn (human) are both diaphragmatic in nature (Manning, 1977). The sternomastoid and gastrocnemius muscles are not as important in maintaining life of the newborn rat and would partially explain the immaturity of these two muscles at birth.

It is interesting to note that the maternal exercise of the SEV intensity did not appear to assist in advancement of

neonatal diaphragm muscle. Maternal exercise has been shown to increase fetal breathing movements in the human (Marsal *et al.*, 1979). It is not known whether this phenomenon occurs in the fetal rat or whether increased useage of this muscle would lead to a more developed muscle at birth. The SEV exercise intensity, however, did not appear to affect the development of this muscle when the neonates of the PR group were compared to the PC group. Perhaps the alterations (if they exist) are more subtle than the changes that may occur using the histological and histochemical parameters studied. Alternative methods of analysis such as ultrastructural or biochemical analysis may manifest changes.

Maternal exercise of the SEV intensity did not appear to affect development of fetal skeletal muscle when cranial and caudal (sternomastoid as opposed to gastrocnemius) muscles were compared. Both the PC and PR neonates showed characteristic newborn rat skeletal muscle. This is important to consider in addition to the results of the organ weight values for if 'hypoxic' reactions were occurring in the fetuses of the PR-SEV group, such as the shunting of fetal blood to favour the vital organs (heart and brain), then perhaps the susceptibility of skeletal muscle to nutritive changes would also be reflected in this reaction. The fetal shunting of blood from the caudal to the cranial area and the resulting diminished blood supply to the caudal area may have been reflected in gastrocnemius

muscle development. However, not only were the PR neonatal body weight values the same as the neonates of the PC group, but neonatal organ weight values did not differ, and neonatal skeletal muscle in the PR group was typical of newborn rat muscle. These results would lend support to the theory that adaptive mechanisms may have developed along with the fetuses of the PR-SEV group from early in gestation which allowed the fetuses of this group to cope with alterations in fetal environment as a result of maternal exercise.

SUMMARY:

Examination of the literature relating to maternal exercise and fetal outcome often gives rise to conflicting results. It would appear that data pertaining mainly to pregnant mice, guinea-pigs and rats forced to run during gestation would give rise to the following theory:

1. The stress of <u>acute</u> repetitive maternal exercise (without prior training) of high intensity levels during mid-gestation appears to alter the protective mechanisms usually present in normal pregnancies by changing the fetal environment. These alterations in the fetal environment become manifest in fetal growth and development. Smaller fetal body weights, organ weights, delay in skeletal ossification and an increase in fetal mortality rates have all been associated with high

intensity levels of acute repetitive maternal exercise during mid-gestation.

2. Prepregnancy training or training in early pregnancy and familiarization of the animals to running may reduce the teratologic effects associated with acute repetitive maternal exercise. Adaptive mechanisms may develop concomitant with fetal and placental growth, to protect the fetus and to ensure only minimal alterations occur in fetal environment. Also, a protected fetal environment would perhaps only elicit minimal changes in fetal growth and development, even at the more severe intensities of maternal exercise throughout gestation.

The neonatal data of the present study would support this theory. Even the most severe maternal exercise intensity did not reduce neonatal body weight values, organ weight values or seem to affect histologically, or histochemically, the neonatal skeletal muscle tissue taken from different parts of the body. It would appear that the fetal environment was somehow protected from severe alterations because no changes were observed in fetal outcome. The protective devices involved in fetal development associated with maternal exercise have yet to be determined but they may exist as anastomotic channels, larger placental areas or in the surplus of blood returned

to the fetus after maternal exercise. One other point that should be mentioned is that perhaps alterations in the neonates of the maternal rats that ran throughout pregnancy may be manifested more subtly in ultrastructural or biochemical analysis.

MATERNAL DATA:

As the previous discussion has suggested, the neonates born to the maternal rats that exercised throughout pregnancy may have been 'spared' harmful effects during prenatal life by the development of adaptive mechanisms. This theory is intriguing especially when considering the maternal data found in the present study. The maternal results showed no significant differences between the maternal rats that ran the MILD exercise intensity throughout gestation and the pregnant control group, although weight gain values during pregnancy were smaller in the PR group. As the intensity of exercise increased, such as that undertaken by the MOD and SEV maternal rats, these reductions in weight gain values were found to be significantly different from control values by the mid-gestation of pregnancy, reaching values of 33.9 g in the MOD experiment and 38.1 g in the SEV experiment by the end of gestation. This is interesting especially when the similar values for average litter weight were considered, as these results may suggest alterations to the exercising maternal system.

The results of the present study supported the similar results of Wilson and Gisolfi (1980) who also reported a significant decrease in maternal weight gain during pregnancy in their trained (seven week prepregnancy training) pregnant rats that ran throughout pregnancy. By the end of gestation these trained pregnant rats weighed 47 g less than their pregnant control group. The offspring born to the trained pregnant group in Wilson and Gisolfi's (1980) study did not exhibit any differences in body and tail dimensions or in organ weights when compared to their control values. It would seem that perhaps mechanisms exist to protect the fetus whilst affecting the maternal system represented by diminished maternal weight gain values. This theory will be discussed in detail later, but two important points should be mentioned.

The first point to consider is that the maternal data in the present study showed no delay in conception for any of the levels of exercise even though the animals had just completed a two week progressive running program. This is interesting to note because in human studies, ammenorhea and disrupted menstrual cycles may be associated with long-term strenuous levels of exercise and training. The effects of exertion on reproductive function, however, have not been completely resolved (Cumming and Belcastro, 1982; Cumming and Rebar, 1983), although there does not appear to be a link between exercise intensity and conception delay (Physician and Sportsmedicine, 1974). The results of the

present study with rats would tend to support this finding.

The second point of interest is that the significant difference found between the maternal rats that ran the MOD and SEV exercise intensities and their control groups, was not present at the beginning of gestation as no difference was found between these groups in body weight values on the day of conception. These body weight values were also not significantly different from those values found in the normal control group of rats. This is particularly important because the main experimental groups (PC and PR) for the MOD and SEV exercise intensities had just completed the two week prepregnancy running program. This would indicate that the two week progressive exercise program did not cause a significant reduction in body weight in the PC and PR groups before gestation. Terada (1974), however, found a significant reduction in body weight gain in mice that were trained over a four week period before pregnancy when compared to controls. A prepregnancy running program longer than two weeks may have elicited a significant change in the body weights of the exercising rats in the present study.

It was interesting to observe that in the SEV exercise experiment the non-pregnant NPR group that undertook the same progressive treadmill running program as the PR-SEV group was also not significantly different from the C group at the end of these two weeks. A significant reduction in average body weight gain was found, however, in the PR-SEV (compared to PC-SEV) and the NPR-SEV (compared to C) groups

on day 11 of the maternal running program. It would appear that the SEV exercise intensity did not manifest a significant reduction in weight gain until close to four weeks after initiation of the exercise (two weeks progressive treadmill running plus 11 days of running into the 21 day pregnancy time period) in both the pregnant (PR) and non-pregnant (NPR) groups. Expressed in this way, the approximate four week time period of running in which a significant reduction in body weight was found in both PR and NPR groups, would agree with the findings of Terada (1974).

In the present study, the effects of exercise did not become apparent until the second third of pregnancy in the PR-MOD and PR-SEV groups. Terada (1974) found that exercise during mid-pregnancy in both the trained (four week prepregnancy training) and untrained (no exercise before pregnancy) groups of mice also interfered with maternal body weight gain. He reported that the exercising maternal mice significantly decreased average daily food and water intake levels when compared to control intake values. The decreased maternal body weight gain in his group of mice may have been attributed to the disturbance found in caloric intake levels (Terada, 1974).

The daily amount of food and water ingested by the pregnant rats in the present study was not determined, but a diminished caloric intake may in part explain the significant reduction in maternal weight gain in the PR-MOD

and PR-SEV groups. Perhaps the intensity of the MILD exercise was not of sufficient intensity to cause alterations in maternal eating habits.

However, diminished maternal intake levels without maternal exercise produced a reduction in fetal body weight, as shown by Lederman and Rosso (1981) who reported that food restriction of 50% during pregnancy showed a decrease in fetal size in the rat. In spite of the adverse fetal affect, alterations in maternal net body weight and body composition were similar in pregnant and non-pregnant rats during food restriction, indicating that the fetus was not capable of parasitizing the tissues of an undernourished maternal system to maintain normal growth (Lederman and Rosso, 1981). Since fetal body weights were not affected in the present study, reduction in maternal intake levels to the extent reported by Lederman and Rosso (1981) would not seem likely. It is interesting to note that in Terada's (1974) study, where the maternal intake was decreased because of maternal exercise, with a concomitant drop in maternal body weight gain, the fetuses were also reported to be of smaller weight even in the trained group. The training intensity of Terada's study may have caused the maternal mice to ingest less than 50% (the value was not given) of the ingested values of control mice, which would help explain the reduced fetal weights.

Another interesting factor in Terada's (1974) study besides nutrition levels in the pregnant mice, was that the

prepregnancy training program for his trained mice was not continued throughout pregnancy but rather this group was forced to run during mid-gestation only, along with his untrained group. When his two groups (trained and untrained) were compared, the training before pregnancy reduced the teratologic effects found in the fetuses of the untrained group but fetal body weight values in the trained group were still decreased. In the present study, however, although body weight gain was significantly reduced in the exercising maternal rats (MOD and SEV), no difference was found in neonatal body weight values. This would indicate that exercise throughout gestation in the pregnant rat, either did not reduce caloric ingestion below 50% of normal values, or, maternal exercise in early pregnancy is an important factor in sparing the fetus by allowing it to parasitize the maternal tissues to maintain normal fetal development, perhaps along with the growth of adaptive mechanisms.

One important point to consider in attempting to explain decreased maternal body weight values is that training in non-pregnant female rats has been shown to enhance lipid oxidation and the sparing of carbohydrate usage during prolonged exercise (Divine-Patch and Brooks, 1980). The relatively lower stress on trained animals at given speeds of treadmill running has been shown to favour fat catabolism and even during hard exercise, trained animals maintained blood glucose at levels comparable to those seen at rest (Divine-Patch and Brooks, 1980). The

reduced oxidation of carbohydrates and the enhanced use of other substrates such as fatty acids in trained rats has also been suggested by other groups of researchers (Fitts *et al.*, 1974; Baldwin *et al.*, 1975; Davies *et al.*, 1981; Brooks and Donovan, 1983; Donovan and Brooks, 1983). This mechanism of carbohydrate sparing may also be present during maternal exercise in trained rats. This carbohydrate-sparing mechanism may have occurred in the present study and perhaps maternal body weight values were found to be decreased in the PR groups because of a reduction in maternal body fat, since more of this substrate may have been used for fuel by the maternal system during exercise.

The maintenance of adequate glucose levels in trained pregnant animals, even during hard exercise may be important in maintaining normal fetal growth. Glucose is a major substrate utilized by the fetus for energy and growth (Young, 1976), and perhaps the use of this substrate during hard intensity maternal exercise in the untrained animal for maternal fuel may cause fetal growth retardation. This may in part explain the reduced maternal body weight values coupled with smaller fetal birth weights in Terada's (1974) study. The present study would also support this theory in that exercise <u>throughout</u> pregnancy decreased maternal body weight values without affecting fetal growth.

The significant decrease in maternal body weight gain values continued when the maternal rats in the MOD and SEV experiments were analysed after giving birth. The method of

statistical analysis for these postpartal results must be interjected. When using the ANOVA to compare postpartal maternal data, there was no significant interaction effect when the three levels of maternal exercise were compared. However, when each of the three experiments was evaluated by comparison of the paired PC and PR values (by the use of a paired Student's t-test), significant differences were found between the paired PC and PR groups for the MOD and SEV experiments in postpartal weight gain (postpartal weight minus weight on day of conception) values. It may be argued that since the ANOVA pools the mean values, the significant results from the paired data in each intensity of exercise may be lost.

In accordance with this, the significant differences in maternal body weight gain reported during pregnancy in the MOD and SEV experiments were sufficient to be detected by the paired Student's t-test and the more powerful ANOVA. After birth, however, the significant differences found between the MOD and SEV paired PC and PR groups were reduced sufficiently to miss detection by the more powerful ANOVA, but these values were still sufficiently different to achieve significant values when analysed by the paired Student's t-test. Observation of these large consistent differences in postpartal weight gain values in the paired PC and PR groups in the MOD and SEV experiments, would indicate that perhaps the results of the paired Student's t-test are an important result and should not go without

mention.

It is important to discuss the significant reduction in body weight gain in the MOD and SEV experiments found at the end of gestation and postpartally. To explain further the reduction in maternal body weight at the end of gestation, illustrations of average maternal body weight gain values will be compared for the PC-MOD and PR-MOD groups in Figure 5A. The same average values will be shown for the PC-SEV and PR-SEV groups in Figure 5B.'

The average weight gained during pregnancy for PC-MOD, PR-MOD, PC-SEV and PR-SEV, can be divided into the following components:

1. a maternal component (average postpartal weight minus average weight on the day of conception) which includes uterus, mammary tissue, blood volume, extravascular and extracellular water, and fat. The placentae would also be included in this component because the maternal rat ingests the placentae after giving birth.

2. a fetal component represented by the average total litter weight value for each group. This component would also include a small and probably insignificant part of the umbilical cord which remains on the neonate at birth.

'Figures 5A, 5B and 6 are included in the discussion for clarity in the explanation of the results.

FIGURE 5A. Average maternal body component analysis of total pregnancy weight gain (expressed in g) for MOD experiment.

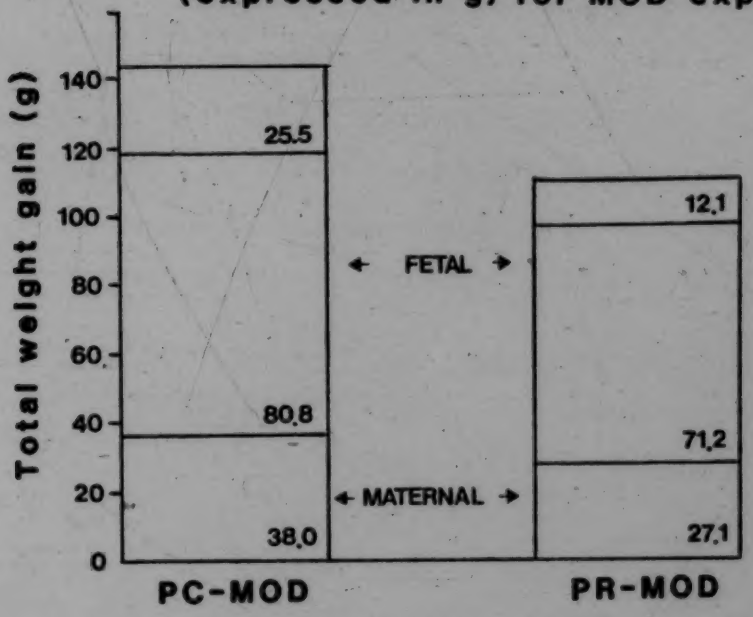
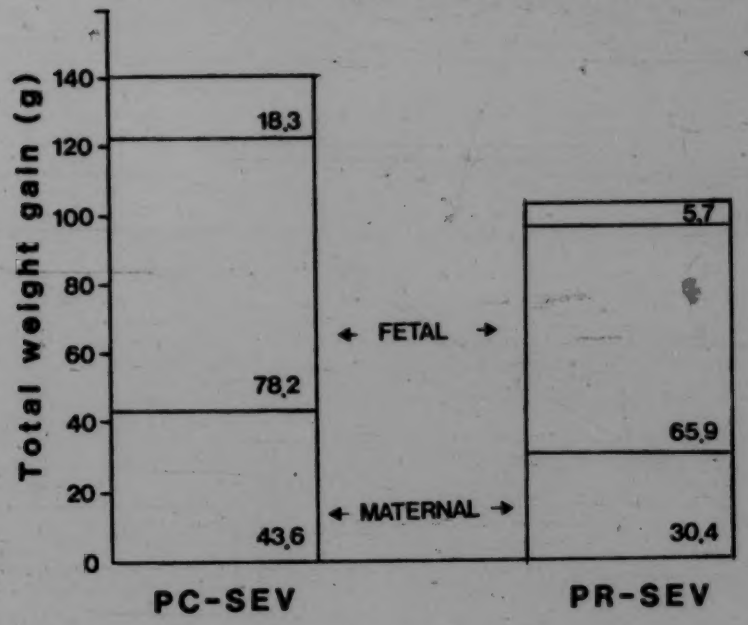


FIGURE 5B. Average maternal body component analysis of total pregnancy weight gain (expressed in g) for SEV experiment.



3. a remnant component which would include the products of conception and/or maternal tissues lost at birth. This third component includes amniotic fluid and membranes from the conceptus and would also be composed of maternal blood and fluid loss that normally occurs during parturition.

The components of maternal weight gain during pregnancy in the present study were based on a similar scheme used by Martin (1980), who analysed components of average human maternal weight gain by term. He divided the weight gained into two main components; products of conception which included, fetus, placenta, membranes, and amniotic fluid; and a second component of an increase in maternal tissues. The second component included increases in uterus, breasts, blood volume, extravascular and extracellular water, and fat.

By dividing the maternal data at the end of gestation into components, it can be seen from Figure 5A that proportionately the PR-MOD group had a significantly smaller maternal component than the PC-MOD group. In contrast, the fetal components were very similar. The difference in maternal components could be due to a difference in uterine weight. This seems unlikely, however, because the fetal components were similar. The more likely explanation would be in the deposition of maternal fat because training has been shown to enhance lipid oxidation (Divine-Patch and

Brooks, 1980), which may reduce fat stores. However, differences which may occur in blood volume, including body water cannot be overlooked. Lederman and Rosso (1981) analysed the average carcass composition of pregnant (minus the conceptus) and non-pregnant rats on day 21 of pregnancy. They reported that on day 21 pregnant rats were composed of an average of 167.1 ±2.9 g of water, 40.7 ±4.4 g of fat and 60.0 ±1.9 g of lean dry tissue. Non-pregnant rats on that same day were composed of an average of 149.5 ±2.4 g of water, 28.2 ±2.2 g of fat and 57.4 ±1.0 g of lean dry tissue. The major difference between pregnant and non-pregnant rats on day 21 of gestation in the maternal component was in weight gained due to fat (12.5 g) and weight gained due to water retention (17.6 g). The average values of Lederman and Rosso (1981) would indicate that normally, pregnant rats gain a substantial amount of fat and water. The PR group in the present study, however, may be deficient in either fat deposits or blood volume. These factors will be discussed shortly.

The fact that the maternal rats ingested the placentae is an important point to also consider. Perhaps, the maternal running rats ate smaller placentae, but smaller placentae usually accompany smaller fetal body weights (Nelson *et al.*, 1983) and the neonatal body weight values in the PR-MOD group were not different from controls. It would not seem likely then that the difference found in the maternal components in the MOD experiment would be due to

the ingestion of smaller placentae in the PR group. This difference in the maternal components may be mainly due to maternal fat stores and blood volume. However, the other contributors to this component may also play a role.

The reduction in the third component found in Figure 5A is intriguing. It seems that the MOD exercise intensity also affected the products of conception (amniotic fluid and membranes) and/or the maternal tissues (blood and fluid) lost at birth. This reduction found in the PR-MOD group for the third component is difficult to explain.

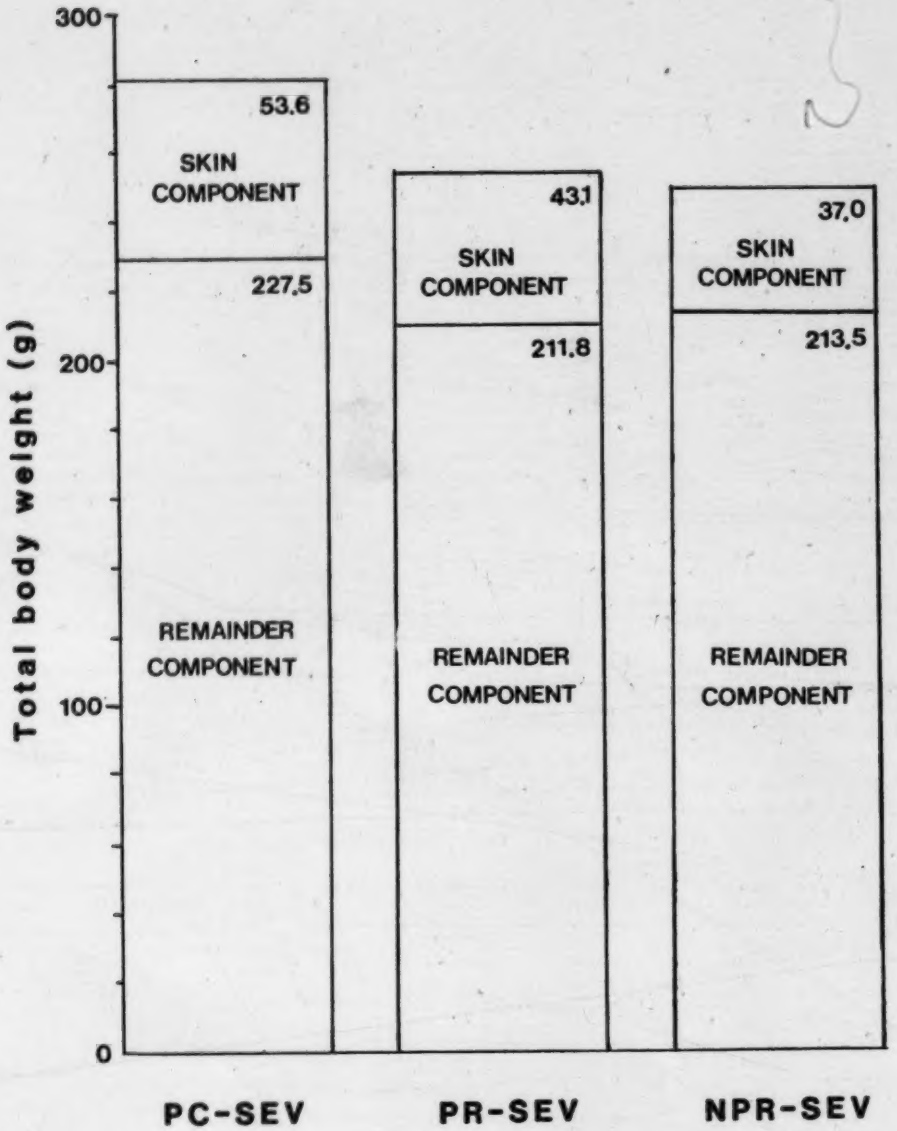
The average component values from the SEV experiment found in Figure 5B, supports the findings found in Figure 5A. However, the effects of the maternal exercise found in the MOD experiment relating to the third component appear to be augmented further in the PR group of the SEV experiment. This is interesting to consider, especially when the differences between maternal components of the PC and PR groups for both experiments are similar and the fetal components are not different. However, the third component appears to be markedly reduced in the PR-SEV group. If this reduced third component indicates decreased amounts of amniotic fluid in the PR-SEV group, then perhaps fetal deformities and abnormalities associated with oligohydramnios might have been observed. Since fetal abnormalities were not evident, it is possible that this severe level of maternal exercise may have decreased the amount of amniotic fluid, but not to a possible critical or

threshold point where abnormalities in fetal development would occur. Perhaps the severe exercise intensity reduced maternal blood volume sufficiently to reduce the amount of blood loss at birth, but not enough to cause critical alterations in the amount of amniotic fluid during gestation. Sufficient maternal blood volume is an important factor during gestation because maternal hypovolemia has been linked to oligohydramnios (Longo, 1983). Adaptive mechanisms may have developed early in pregnancy to protect the fetus from diminished amounts of amniotic fluid perhaps at the expense of maternal blood volume. This reduced third component needs further investigation.

The maternal component in the SEV exercise experiment was analysed one step further in that the postpartal body components (skin weight and carcass remainder weight) were examined. The significant differences found in the maternal components between the PC and PR groups proved to be interesting. In figure 6 the actual average weight in grams for the carcass components are presented for the PC-SEV, PR-SEV and the non-pregnant NPR-SEV groups. The skin component consisted mainly of skin (fur), subcutaneous fat (an important large fat storage area in the rat; Galletti and Klopper, 1964) and mammary tissue. The carcass remainder component consisted of muscle tissue, abdominal contents, thoracic contents, internal fat depots, and tail.

In comparing the skin component values (figure 6), it was interesting to note that the PC group had 10.5 g more of

FIGURE 6. Average postpartal body component analysis (expressed in g) for SEV experiment in maternal rats compared to NPR.



this component than the PR group, although both of these groups had just given birth. Furthermore, the PR group had an average of 6.1 g more skin component than the non-pregnant NPR group.

In comparing the remainder component, the PC group had 15.7 g more carcass remainder weight than the PR group, even though both PC and PR groups had just given birth to similar neonates. The difference in this body weight component would probably not be due to the weight of the uterus, nor would it seem likely that the PC group ingested larger placentae, since the neonates were of similar size. It also does not appear likely that the PC group would have more muscle and bone because they did not run. However, the PC group may possibly have more internal fat deposited or more blood volume as indicated by the results of Lederman and Rosso (1981) in their study of pregnant rats. The smaller amount of adipose storage found in the PR group would support the theory that during training, fatty acid oxidation was enhanced while perhaps sparing maternal glucose for fetal usage. The findings of diminished adipose tissue found in the PR group is further supported by the fact that the carcass remainder weight values for the PR and NPR groups are almost identical. This similarity in remainder weight does not account for the heavier uterine tissue found in rats just after parturition. This factor is difficult to interpret.

The 6.1 g difference in skin component found between the PR and and NPR groups may be due to the glandular tissue of the mammary glands or perhaps to the attempt of the PR group to store fat subcutaneously for lactation. In either case the reduced amount of maternal adipose tissue may prove harmful after birth, in that insufficient storage of fat in the maternal system or underdeveloped mammary tissue may give rise to inadequate milk production to feed the nursing neonates. This would perhaps help to explain the findings of Wilson and Gisolfi (1980) who reported a significantly higher number of neonatal deaths attributable to maternal neglect and maternal cannibalism in their trained maternal rats who also ran during pregnancy, during a postpartal observation period of 28 days. Wilson and Gisolfi (1980) also reported a significant reduction of 47 g in body weight gain for this group at the end of pregnancy. The maternal rats in their study were not analysed postpartally but if this 47 g deficit in weight gain was due to insufficient fat storage or underdeveloped mammary glands, then it is possible that insufficient milk production might cause these maternal rats to cannibalize their young or to neglect them. In the present study, no maternal neglect was observed, but, had the neonates not been sacrificed within 20 hours of birth, perhaps the maternal neglect and the cannibalism as seen in Wilson and Gisolfi's (1980) study would also have been observed. Maternal postpartal body component analysis in running animals needs further investigation.

The data reported for normal pregnant rats indicated that body weight gain values (in addition to the conceptus) are composed predominantly of fat deposition and water retention. Both of these factors appear to be affected when the maternal results of the present study were examined. It would appear that perhaps the fetuses are spared at the expense of the maternal system in either storage of adipose tissue for lactation or maternal blood volume or both, especially at the more severe levels of maternal exercise.

SUMMARY:

The maternal data added an interesting finding to the theory presented in the neonatal section. It would appear that protective mechanisms may exist to spare the fetus during maternal exercise at the MOD and SEV exercise intensities at the expense of the maternal system, especially at the SEV level of exercise. This was particularly evident in the significant reduction in maternal weight gain found for both groups of maternal rats (PR-MOD and PR-SEV) at the end of gestation and postpartally. The alterations found in the maternal system probably exist as insufficient adipose tissue storage for lactation especially at the more severe levels of maternal exercise. Although adaptive mechanisms that may occur during training may serve to protect the fetus, such as enhanced fatty acid oxidation and glucose sparing, the deletion of adipose tissue stores may perhaps cause problems for the

neonates in postnatal life. More research is needed in the area of maternal exercise and its effects on the mother and the developing fetus.

CONCLUSIONS AND RECOMMENDATIONS

SUMMARY

The purpose of the present study was to examine the effects of three intensities of maternal aerobic exercise (mild, moderate and severe) throughout gestation on the fetus in the rat. The effects of maternal exercise on fetal growth and development were analysed by newborn number per litter, observation of gross superficial abnormalities, newborn gross body weight and newborn organ weights. At the microscopic level, neonatal skeletal muscle was chosen for analysis. The effects of maternal exercise on the gross morphology of the maternal rat were represented by maternal weight gain during pregnancy, maternal postpartal weight and maternal postpartal skin (including subcutaneous fat and mammary tissue) and the remainder component analysis.

CONCLUSIONS:

Within the limitations of this study the following conclusions were presented:

1. Maternal aerobic exercise of mild, moderate and severe intensities undertaken throughout gestation did not affect fetal development.

2. The maternal aerobic exercise of the mild intensity showed no apparent effect on the maternal rats.

3. The maternal aerobic exercise of the moderate and

severe intensities caused a reduction in maternal body weight during pregnancy.

4. The maternal aerobic exercise of the severe intensity caused a reduction in the maternal skin component (including subcutaneous fat and mammary tissue) and the maternal remainder component when analysed postpartally.

5. The fetuses of the maternal rats who underwent the moderate and severe aerobic exercise intensities throughout pregnancy were somehow 'spared' at the expense of the maternal system.

RECOMMENDATIONS:

1. More research is needed to determine whether adaptive mechanisms do exist as a result of training. The recommended areas of study would be:

   a. identification of anastomotic channels in the uterine blood supply of trained pregnant rats

   b. assessment of placental weight, diffusing capacity, and capillarization in trained pregnant rats

   c. measurement of the amount of amniotic fluid present in the fetuses of trained maternal

rats just prior to birth.

2. Additional analysis is required in the body components of trained pregnant rats during gestation and postpartally. Analysis should be centred mainly on the following areas:

   a. measurements of internal and subcutaneous fat depots in the trained pregnant rat

   b. measurement of the pregnant uterus before and after parturition in trained rats

   c. assessment of maternal blood volume just prior to birth in trained pregnant rats

   d. analysis of mammary tissue in trained rats before and after parturition.

3. Further study of the neonates of trained maternal rats with regard to growth rate and learning ability is required.

4. Additional assessment of the behaviour of trained maternal rats towards their offspring until the time of weaning is also important.

REVIEW OF LITERATURE

Literature reporting on the effects of maternal exercise on fetal growth and development is often conflicting. An attempt has been made to sort out the discrepancies in the literature and to relate these discrepancies to various factors which may facilitate comparisons of the results of the current research situation, pertaining to the area of maternal exercise.

In reviewing the literature these discrepancies appear to be due to three major factors:

5. the use of different animal species for experimentation in maternal exercise,

6. the familiarization and/or training of the maternal animal before pregnancy, and

7. the use of different exercise intensities.

There are three other factors which should not go without mention:

1. the techniques used to 'prompt' the maternal animal to exercise,

2. the use of invasive techniques to monitor the maternal and/or the fetal systems, and

3. deprivation of the maternal animal of food and water for any length of time before maternal exercise.

Each of these factors will become evident as the presentation of the literature proceeds. The literature review will be presented by separating the research reported into animal species used for experimentation.

There are five major species of animals used for experimentation in the area of maternal exercise. These species include: rats, mice, guinea-pigs, sheep (and Pygmy goats), and the human. The data pertaining to maternal exercise in each of these species will be presented. A section on the effects of training on non-pregnant rats will be examined first.

EFFECT OF TRAINING ON NON-PREGNANT RATS:

The laboratory rat has been used frequently to study chronic metabolic adaptations to exercise (Shepherd and Gollnick, 1976). Many different techniques have been used, however, to train rats and this review centres mainly on the training procedures and resulting adaptations that occurred in trained running female rats.

Fitts *et al.* (1974) exercised four groups of rats for fourteen weeks. At the end of this program, the rats were running at a treadmill speed of 31 m/min, up an 8° incline, 5 days/week at either 10, 30, 60, or 120 minutes/day. The authors also compared different training indices such as mitochondrial markers in gastrocnemius muscle, and glycogen depletion from leg muscles. The results of these tests indicated that a good correlation existed between the level

of training, the respiratory capacity of exercising muscles, and endurance. They concluded, that as the level of training increased, the mitochondrial content of the exercising muscle increased, and this appeared to reduce the amount of carbohydrate utilized during intense submaximal exercise.

The suggestion that training may increase endurance by involving a mechanism which appeared to spare glycogen utilization has been supported by other researchers (Baldwin *et al.*, 1975; Divine-Patch and Brooks, 1980; Davies *et al.*, 1981; Brooks and Donovan, 1983; Donovan and Brooks, 1983). Baldwin *et al.* (1975) demonstrated a slower utilization of glycogen in skeletal muscle in exercise-trained female rats when compared to untrained control rats. These authors also showed an increase in muscle mitochondria in the muscles of the exercise-trained group. They suggested two major changes occurred because of this adaptation (increase) in muscle mitochondria. The first change was a slower rate of glycogenolysis in the working skeletal muscle. The second alteration was a shift in the carbon source for the citric acid cycle, with more energy being drawn from the oxidation of fat and less energy being derived from carbohydrate in the trained as compared to the untrained state, at the same submaximal rate of work. These authors also suggested another contributing factor to this glycogen-sparing effect might be related to a more rapid increase in blood flow and oxygen delivery to the working muscles in the trained group. Another interesting finding from this study was that liver

glycogen concentration at rest was 75% higher in the trained group of rats when compared to the untrained control group. This increased level of liver glycogen storage may also protect the trained animals by ensuring maintenance of blood glucose levels during prolonged exercise (Baldwin *et al.*, 1975).

Divine-Patch and Brooks (1980) also suggested carbohydrate-sparing occurred in trained female rats when compared to untrained controls. The training procedure used in their study involved five weeks of incremental exercise and one week of daily running for one hour/day at a treadmill speed of 28.7 m/min on a 15% grade (8.5° incline). The untrained rats were accustomized to treadmill running on a 15% grade by 5 to 8 minute exposures, twice/week. The treadmill speed for the untrained rats during these brief exposure times was gradually increased until 28.7 m/min was attained just prior to the testing period. The two groups of rats (trained and untrained) were examined during running at 14.3 m/min on a 1% grade for one hour ('easy exercise') and running at 28.7 m/min on a 15% grade for one hour ('hard exercise'). The results indicated that during the easy exercise test both untrained and trained animals maintained blood glucose values when compared to rest values. During the hard exercise test, however, only the trained animals could maintain blood glucose levels comparable to those values found at rest. The extremely low blood glucose levels observed in the untrained animals occurred after only thirty

minutes of the hard exercise test and may have contributed to the fatigue of these animals (they were not able to run the full hour). The authors suggested that in trained animals, superior capacities for glucose-glycogen sparing, gluconeogenesis, and fat catabolism may be present.

In accordance with this, Davies *et al.* (1981) also suggested the increased endurance observed in their trained animals (when compared to untrained) may have been due to enhanced glycogen-sparing, the basis of which may be increased lipid utilization and/or an elevated capacity for gluconeogenesis. The results of Davies *et al.* (1981) indicated substantially lower respiratory exchange ratios (even at $\dot{V}O_2max$ for their endurance-trained animals), suggesting a significantly higher contribution of lipids to energy metabolism in this group. These authors also reported that the trained animals obtained about 34% of their total energy from lipids during endurance tests, whereas lipid utilization in control animals was only 10%. These values indicated that the trained group of rats utilized 3.3 times more lipid than controls and this factor may postpone glycogen depletion, thereby increasing endurance (Davies *et al.*, 1981). The training program for the trained group of rats was 10 weeks in duration, commencing treadmill speed was 26.8 m/min on a grade of 8.5° for 20 minutes. Thereafter running time increased 5 minutes/day, until by the fifth week, the rats ran 120 minutes/day for 5 days/week. For the remaining 6 weeks the animals maintained this intensity. The

untrained control group was exercised at the same treadmill speed for 10 minutes every third day for the entire 10 weeks.

In two more recent studies (Brooks and Donovan, 1983; Donovan and Brooks, 1983) the effects of endurance training (treadmill speed was 29.4 m/min up a 15% grade for up to 120 minutes/day, 5 days/week) on glucose metabolism was examined. Untrained control animals were exposed to treadmill running for 10 minutes once/week. The animals were examined at rest, during easy exercise (treadmill speed of 13.4 m/min, 1% gradient), or during hard exercise (treadmill speed of 26.8 m/min, 1% gradient). The results showed that $\dot{V}O_2max$ in the endurance-trained rats was increased by 13% over the aerobic capacity values found for the control group, demonstrating a significant training effect (Brooks and Donovan, 1983). Examination of blood glucose values revealed that trained animals were better able to maintain glucose homeostasis during hard exercise. In addition, the major contributing process to carbohydrate metabolism in muscle was not glucose uptake from the blood but rather muscle glycogenolysis which would indicate that during rest or exercise the primary carbohydrate source is muscle glycogen. In the trained rats improved glucose homeostasis occurred which resulted from increased conversion of lactate to glucose and reduced lactate oxidation during hard exercise (Donovan and Brooks, 1983). Also, trained animals exhibited a greater lactate metabolic clearance rate during

the hard exercise, attributable to a lesser autonomic response in trained animals. Elevated blood glucose levels in trained animals and greater conversion of lactate during hard exercise were consistant with the maintenance of splanchnic blood flow. In contrast, the stress of hard exercise in untrained animals may trigger an autonomic response that shunted splanchnic blood away from the gluconeogenic organs (liver and kidney) towards the working muscle, limiting the capacity for release of glucose from the liver (Donovan and Brooks, 1983).

The 'glucose-sparing' and enhanced lipid oxidation found in trained animals may actually decrease body weight due to a decrease in body fat. Kral *et al*. (1974) found that training caused a decrease in body weight, body triglyceride and adipose cell size. They suggested that physical training may lead to an adaptation of adipose tissue metabolism with an enhanced insulin sensitivity. The trained animals were male rats engaged in an intensive training program of swimming.

PREGNANCY AND EXERCISE IN THE RAT:

Atherton *et al*. (1982) recorded body weight changes in pregnant Sprague-Dawley rats and compared these values with paired non-pregnant controls. Differences in body weight values between these two groups of rats were significant from day 5 onward. On day 21 of gestation the pregnant rats had gained approximately 53% of total weight from day 2 of gestation compared to an increase of only 8% reported for

the non-pregnant controls. Lederman and Rosso (1981) actually measured the average carcass composition of pregnant (minus the conceptus) and non-pregnant rats on day 21 of pregnancy. Carcass analysis demonstrated that on day 21 pregnant rats were composed of an average of 167.1±2.9 g of water, 40.7±4.4 g of fat and 60.0±1.9 g of lean dry tissue. Non-pregnant rats on that same day were composed of an average 149.5±2.4 g of water, 28.2±2.2 g of fat and 57.4±1.0 g of lean dry tissue. The major contributing factors to the differences in weight gain between pregnant and non-pregnant rats (besides the conceptus) were the amount of fat deposited and maternal body water (Lederman and Rosso, 1981). Atherton *et al.* (1982) reported a significant increase in plasma volume from day 6 of gestation onward in pregnant rats (about 30% higher close to term) which would support the larger body water values found in the pregnant rats analysed in the study by Lederman and Rosso (1981) when compared to controls.

Normal pregnant rats give birth to between 9 and 16 offspring per litter (Atherton *et al.*, 1982). Several authors have reported similar values for normal pregnant rats, an average of 8.85±2.56 neonates per litter (Buhrdel *et al.*, 1978); a range of 9 to 12 offspring per litter (Parizkova, 1975); Parizkova and Petrasek, 1978); and an average value of 9.5±3.5 neonates per litter (Wilson and Gisolfi, 1980). Vorherr (1982) reported rats normally have a mean of 10 offspring per litter. In this regard Vorherr

(1982) reported that the total birth weight of a litter should be about 20% that of maternal weight and with an average litter size of 10, the birth weight of one pup would be 2% that of the maternal rat (Vorherr, 1982).

The combination of pregnancy and exercise on fetal development does not appear well documented for the rat. Parizkova (1975) examined the microstructure of the male offspring of female rats exercised during pregnancy at 100 days of age after birth. The exercise protocol was a treadmill speed of 14 to 16 m/min for one hour daily throughout pregnancy. This protocol was considered to be of mild aerobic character (Parizkova, 1975). The results indicated that total body weight values of the male offspring of the rats who ran throughout pregnancy did not differ from controls either at birth nor at 100 days of age. The heart weight, however, from the male offspring of the exercised maternal rats was significantly higher at 100 days of age. The microstructure of the heart also showed a significant increase in the number of capillaries and muscle fibers/mm^2, a significantly higher heart capillary:muscle fiber ratio and significantly shorter distance for diffusion in heart muscle in the male offspring of maternal running rats. Parizkova (1975) concluded that during the prenatal period the developing fetal heart appears to be susceptible to the stimulus of maternal exercise.

In a later study, Parizkova and Petrasek (1978) examined the effects of maternal exercise on offspring lipid

metabolism. Again, mild aerobic exercise was selected for study (14-16 m/min, one hour/day, throughout gestation). The results suggested that regular exercise during pregnancy had a marked influence on selected indicators of lipid metabolism in the liver of both male and female offspring during postnatal examination.

Corbett *et al.* (1979) examined the skeletal muscle metabolism in the offspring of trained maternal rats (no training protocol was given). The authors sacrificed offspring at 18 days of age. The results indicated that endurance training prior to, or during pregnancy, had no effect on glycolytic, oxidative or contractile properties of skeletal muscles (soleus, plantaris and gastrocnemius) examined from the offspring (Corbett *et al.*, 1979).

Wilson and Gisolfi (1980) analysed male offspring (between 45 and 65 days of age) of trained maternal rats, with special emphasis on the cardiovascular system. Their study consisted of four groups of rats: animals who ran for seven weeks at 35 m/min up a 1% grade, one hour per day for six days before, but not during gestation (T-NT); animals who also ran this prepregnancy training program for seven weeks before gestation and then ran 32 m/min up a 1% grade for seven days/week for one hour/day throughout pregnancy (T-T); animals who only ran at a treadmill speed of 16 m/min up a 1% grade, for one hour/day, seven days/week during pregnancy (NT-T); and a control group of animals who did not run (C). The measurements taken in the male offspring

included $\dot{V}O_2max$, coronary blood flow under hypoxic (10% O_2, 90% N_2) or normoxic (room air) conditions, myocardial capillary density (histological preparation), cardiac muscle fiber:capillary ratio, and measurement of organ (heart, kidney, spleen, adrenal) and body weights. The results indicated that body dimensions, organ weight values and $\dot{V}O_2max$ were not significantly different between the offspring in any of the groups. Wilson and Gisolfi (1980) also reported a 'trend' for heart:body, left ventricle:heart and right ventricle:heart weight to be highest in the offspring of NT-T and T-T groups. The authors found no anatomical difference in total capacity of myocardial capillaries in the offspring of any of the experimental groups. This result would disagree with the findings of Parizkova (1975) who did find a significant difference in the ratio of heart capillary:fiber in the offspring of trained maternal rats.

Wilson and Gisolfi (1980) also analysed the effects of training on the maternal rat. They examined the cytochrome oxidase activity in soleus muscle, the left ventricle:heart weight ratio and max $\dot{V}O_2$. The results indicated that the T-T group had significantly higher max values than the C group at the end of pregnancy. Pregnancy did not change the training effect on oxidative enzyme activity and heart:body weight ratios, in that a two-fold increase in cytochrome oxidase activity was found in the soleus muscles of T-T and a 1.5 fold increase in the NT-T animals compared with

controls. These results were consistent with physiological changes that occur with different intensities of training. One interesting finding in the maternal data was that the T-T group gained only 57 g at the end of pregnancy, compared with 103, 105 and 78 g for the C, T-NT, and NT-T groups, respectively. An increase in neonatal mortality was also found in the T-T group perhaps due to maternal neglect and maternal cannibalism, both of which were observed in their study. The authors concluded that mild to heavy maternal exercise did not influence $\dot{V}O_2max$ or myocardial structure in male offspring and that high intensity exercise (80-88% $\dot{V}O_2max$) was associated with an increase in offspring mortality during the first 28 days postnatally.

The reduction in maternal weight gain in the T-T group in Wilson and Gisolfi's (1980) study is interesting to consider, especially since training may diminish body fat stores (Kral *et al.*, 1974). Wilson and Gisolfi (1980) did not attempt to expand upon the reasons for the increased offspring mortality found in the high intensity maternal exercise group. Perhaps the maternal neglect and maternal cannibalism they noted as the underlying cause for the offspring death may be related to the diminished maternal weight gain and insufficient maternal fat deposits necessary for lactation. Rosso *et al.* (1981) examined malnutrition and consequent reduced maternal body weight during pregnancy on mammary glands in rats. They reported that when pregnant rats were fed 50% of their normal food intake from day 5 of

gestation onward, the weight of the mammary gland was proportionately more decreased than any other major fat depot. The results of their study indicated that there was a minimal accumulation of fat in the mammary gland of undernourished rats when compared to controls, and also a reduction in the quantity of cytoplasm and cytoplasmic material related to secretory activity (Rosso *et al.*, 1981). The maternal neglect and maternal cannibalism found in the study of Wilson and Gisolfi (1980) may have been associated with underdevelopment of the mammary tissue or insufficient fat in the mammary tissue and consequently insufficient production of nutrients to feed the nursing young, leading to maternal neglect and cannibalism. Further study in this area is required.

PREGNANCY AND EXERCISE IN THE MOUSE:

Terada (1974) investigated the effects of training before pregnancy on the developing fetus and maternal mouse when the pregnant females were forced to run during mid-gestation. Terada used four groups of mice: animals trained for four weeks prior to pregnancy at a treadmill speed of 15 m/min for one hour/day, six days/week, and then forced to exercise during mid-gestation (15 m/min for 30 minutes on the 9th to 16th days of pregnancy) (TE group); animals trained by the same four week exercise program before pregnancy but not exercised during pregnancy (TC group); animals only forced to exercise during mid-gestation (CE group); and animals neither trained nor exercised during

pregnancy (CC group). The results indicated that the four week training program prior to pregnancy, significantly reduced the body weights in the TE and TC groups when compared to the body weight values of the CE and CC groups. Exercise during mid-gestation also interfered with maternal body weight gain in the TE and CE groups and also caused a significant reduction in average daily food and water consumption in the CE group as compared to the values found in the CC group. In the CE group, forced exercise during mid-gestation caused an increase in fetal mortality rate (resorbtion and maceration) and a significant reduction in fetal body weight. The TE group, also forced to exercise during mid-gestation, had small fetal body weight values but did not indicate an increased fetal mortality rate, and both the CE and TE groups showed delay in fetal skeletal ossification. Terada (1974) concluded that the reduction in maternal body weight gain may have been due to a disturbance in caloric intake. Neither training, nor forced mid-gestation exercise affected litter size, or caused malformations. However, forced mid-pregnancy exercise caused a higher mortality rate and lower fetal body weights than the controls. Although the TE group also had smaller fetal body weights, the fetal mortality rate was significantly reduced and perhaps training before pregnancy may have reduced the effects of forced maternal exercise during mid-gestation (Terada, 1974).

PREGNANCY AND EXERCISE IN GUINEA PIGS:

Several researchers from the same research lab have reported on the effects of maternal exercise on the fetus using pregnant guinea-pigs. Gilbert *et al.* (1979) studied the effects of maternal exercise and environmental hypoxia (12% oxygen) on placental diffusing capacity. The exercise protocol was a treadmill speed of 10 m/min up an 8% grade for 15 minutes twice daily for six days and once on the seventh day. The exercise was begun between the 13th and 17th days of gestation. Between 62 and 64 days of gestation (term=67 days) diffusing capacity of the placenta, fetal body weights, fetal heart and brain weights, placental weight and maternal body and heart weights were measured. The results indicated that exercise of this intensity did not cause a reduction in fetal body weights or organ weights. Experimental hypoxia, however, did cause a significant reduction in both fetal body and brain weight values, and an increase in placenta:body weight and heart:body weight ratios. The placental weights of both experimental groups were the same as control values. In the experimentally hypoxic animals, placental diffusing capacity/kg fetal weight was increased markedly. In contrast, the placental diffusing capacity decreased significantly in the exercising animals. The authors concluded that experimental hypoxia significantly decreased fetal and brain weights, while the fetal brain:body and heart:body weight ratios were larger. This suggested that

the fetal brain and heart grew disproportionately larger than the rest of the body which resulted from the 'sparing' of brain and heart by a redistribution of fetal cardiac output favouring these 'survival' organs (Gilbert *et al.*, 1979). Maternal exercise, however, did not alter fetal body weight, or organ weights when compared to controls.

In a later study, Gilbert *et al.* (1981) assessed different intensities of maternal exercise on the fetuses of pregnant guinea-pigs exercised only during pregnancy. The maternal exercise intensities consisted of a treadmill speed of 9.7 m/min up a 6.5% grade at one of five exercise levels. The first level consisted of maternal exercise for 15 minutes, once daily, the second was 15 minutes, twice daily, total time 30 minutes; the third was 15 minutes three times daily, total time 45 minutes; the fourth was 15 minutes, 4 times daily, total time 60 minutes. The final group did not exercise (0 minutes). All the exercising animals ran 5 days/week. The authors assessed fetal body and organ weights, placental weight and maternal body and heart weight near term (on days 63 and 64 of gestation). The results demonstrated a decrease in fetal body weight, placental and fetal kidney weight values as the level (time) of maternal exercise increased. No changes were exhibited in fetal heart and brain weight but the ratio of heart:body weight and brain:body weight ratios were increased at the higher levels of exercise. Fetal body weight values were found to decrease by 30% in the 45 minute/day exercise group and by 11% in the

60 minute/day exercise group. The results also demonstrated a decrease in placental weight and a reduction in placental diffusing capacity/kg of fetal weight. The authors suggested that the fetus was compromised at the higher levels of exercise by a smaller placenta with less diffusing capacity/kg of fetal tissue (Gilbert *et al.*, 1981).

In a more extensive report of the same experiment, Nelson *et al.* (1983) showed that maternal body weight decreased significantly with increasing levels of exercise. It appeared that the maternal body grew at a slower rate during gestation in the exercise groups than in the control group. They determined this by estimating the maternal growth rate which was equivalent to total maternal weight minus fetal and placental weight at term, divided by the number of days of gestation. No alterations were found, however, in maternal heart weight values for the exercising groups. The authors concluded that the decrease in fetal body weight values did not become apparent until a maternal exercise intensity level greater than perhaps thirty minutes per day was attained. Maternal exercise produced detrimental effects on fetal growth and development. These effects were first observed at low levels of maternal exercise by alterations in placental weight and placental diffusing capacity, whilst at the higher levels of exercise, reductions in fetal body weight were observed (Nelson *et al.*, 1983).

Smith *et al.* (1983), also from the same research lab, used stereological techniques to assess the peripheral labyrinth exchange area of guinea-pig placentas from the same execise protocol used by Gilbert *et al.* (1981) and Nelson *et al.* (1983). The data demonstrated that placental diffusing capacity and maternal surface area in the peripheral labyrinth exchange area both decreased as the level of maternal exercise increased. The ratio of fetal weight:maternal weight varied as a function of placental diffusing capacity. In comparing the sum of maternal and fetal placental surface areas for each exercise group with the average fetal weight values for each group, it was demonstrated that the fluctuations found in fetal surface areas may compensate, at least in part, for the decrease found in maternal vasculature, which may prevent changes in fetal weight, at least at the more mild levels of exercise (Smith *et al.*, 1983)

EFFECTS OF PREGNANCY AND EXERCISE IN SHEEP (AND PYGMY GOATS):

Most of the literature pertaining to maternal exercise and the effects on mother and fetus has dealt with pregnant sheep and fetal lambs. The ewe and fetal lamb have been used extensively for invasive research monitoring uterine blood flow, pO_2 levels in both mother and fetus, and alterations in fetal blood flow and fetal metabolism (NICHD Summary Report, 1982). A review of the literature, however,

indicates that there are two groups of researchers, one group showing that there are no alterations in uteroplacental blood flow (UBF) and therefore no alterations in fetal environment as a result of maternal exercise, and another group of investigators, who have found significant changes in fetal growth in association with maternal exercise. These as yet unexplained variations in reports may possibly have resulted from differences in exercise intensities, the physical condition of the animals (trained or untrained), or from other stresses indirectly related to maternal exercise (such as the invasive techniques) (Lotgering *et al.*, 1983A).

Emmanouilides *et al.* (1972) examined the effects of repeated bouts of acute maternal exercise on the sheep fetus in twelve pregnant ewes. In four of these ewes an umbilical artery was deliberately ligated to produce a state of 'fetal distress'. The range in gestational age that surgery occurred was between 100 and 130 days (term=about 147 days). In group A, a catheter was placed into the fetal abdominal aorta and another was placed into the umbilical vein. In group B, 'fetal distress' was induced by ligation of an umbilical artery, and a catheter was inserted into the fetal aorta. A catheter was also inserted into one of the maternal carotid arteries. After eight to thirty-two days following the initial operation, the pregnant ewe was exercised on a treadmill at 2.0-2.5 miles/hour for 30 to 60 minutes. As a result of experimentation, five of the pregnant animals gave

birth to near-term viable offspring, one ewe with a twin pregnancy died of a ruptured uterus after the fourth exercise test, and one ewe spontaneously delivered a stillborn lamb three days after the fifth exercise test (total number of exercise tests were not given). The authors indicated that this type of maternal exercise in the sheep induced decreases in fetal pO_2 values suggesting a diminished oxygen supply to the fetus. This alteration was usually transient and tolerated fairly well in fetuses without umbilical ligation. However, the fetuses (in group B) with ligated umbilical arteries were unable to tolerate maternal exercise as well as the fetuses of group A (Emmanouilides *et al.*, 1972). The authors also postulated that the decreases in fetal oxygenation found during maternal exercise was probably due to a reduction in UBF.

It is difficult to attribute the results of Emmanouilides *et al.* (1972) only to the effects of maternal exercise. The pregnant animals were not trained or even familiarized to the treadmill apparatus. In addition, the animals were all at various gestational ages and the initial exercise testing occurred between 8 and 32 days after surgery which may have altered the results. The fact that Emmanouilides *et al.* (1972) reported five 'near-term' deliveries, one death of a pregnant ewe from a ruptured uterus after four exercise sessions and the birth of a stillborn lamb after five exercise sessions would indicate the stressfulness of the techniques involved; surgery,

invasive catheters, new apparatus (treadmill) and environment. The results of their study may not only indicate the effects of acute maternal exercise but also may demonstrate the reaction of the animal to additional stress.

Longo *et al.* (1978) also reported a significant decrease in fetal descending aortic pO_2 values which fell by 19% in nine chronically catheterized fetal lambs. They exercised their pregnant ewes at a treadmill speed of 43 m/min for 20 to 45 minutes at 10° inclination. Again, no mention was made of either familiarization of the animals to the equipment or to any training protocols. The authors also reported a significant decrease of 59% in uterine blood flow (measured with an electromagnetic flow probe) during maternal exercise. Four ewes were exercised for 15 minutes twice daily during the last three to four months of pregnancy. The fetal lambs of these animals weighed significantly less than controls. The authors concluded that 'moderate' to 'heavy' 'sustained' maternal exercise resulted in significant fetal 'hypoxia' and may cause intrauterine growth retardation (Longo *et al.*, 1978).

Chandler and Bell (1981) also found that acute exercise at 0.7 miles/hour on a 10° incline for 60 minutes produced a reduction in UBF of 36% in pregnant ewes near term. Fetal pO_2 values also declined. These findings would support the results of Longo *et al.* (1978).

In two more recent studies Lotgering *et al.* (1983A and 1983B) (from the same research lab as Longo *et al.*, 1978)

examined acute exercise responses in pregnant catheterized sheep. In a preliminary experiment they determined $\dot{V}O_2max$ in each of the thirteen animals (gestational age was not given) by assessing cardiac output and arteriovenous oxygen content difference during intermittent exercise sessions of ten minutes at increasing levels. Following this, in the main experiment, UBF during three different exercise intensities, was measured. The first exercise intensity lasted for 10 minutes at 70% $\dot{V}O_2max$, the second for 10 minutes at 100% $\dot{V}O_2max$, and the third session was 40 minutes in duration, at 70% $\dot{V}O_2max$. The gestational age at the time of study was 117 to 138 days of pregnancy. The ewes were conditioned to walk on the treadmill at varying speeds for short intermittent periods of time totalling 10 minutes daily for one week before surgery occurred. The pregnant animals were removed from food 12 to 24 hours before surgery, although free access to water was given. During surgery the fetal ascending and descending aorta, superior and inferior vena cava were catheterized. The maternal animal was also catheterized to determine UBF.

The results of Lotgering *et al.* (1983A) showed that UBF was reduced immediately at the onset of maternal exercise and remained significantly below control values for the duration of exercise. A more pronounced reduction in UBF occurred with the heavier exercise sessions (100% $\dot{V}O_2max$) and with exercise of longer duration (70% $\dot{V}O_2max$ for 40 minutes). After the exercise sessions the UBF gradually

reached pre-exercise values within 20 minutes, and after the longer exercise session an insignificant overshoot of 4% above resting blood flow occurred. Plasma volume was also found to decrease an average of 20% during exercise at 70% $\dot{V}O_2$max. An alteration in plasma protein concentration was also found but was only 24% of the change in plasma volume, which indicated, however, loss of both water and proteins from the intravascular compartment. The authors concluded that acute exercise during pregnancy caused major physiological alterations in the maternal animal, such as a reduction in UBF (Lotgering *et al.*, 1983A).

The results of Lotgering *et al.* (1983B) demonstrated considerable temperature increases during maternal exercise. Also, a decrease in fetal arterial oxygen content of 27% and a reduction of 11% in fetal oxygen tension during prolonged maternal exercise (40 minutes at 70% $\dot{V}O_2$max) were reported. There were increased maternal and fetal lactate concentrations during the 40 minute exercise session (70% $\dot{V}O_2$max). During short exercise sessions, however, fetal lactate values did not alter significantly despite elevated maternal values. Glucose and lactate are actively metabolized by both the fetus and placenta (Burd *et al.*, 1975; Charr and Creasy, 1976; Lotgering *et al.*, 1983B) and because of this it is difficult to determine the physiological implications of increased plasma concentrations as a result of maternal exercise. No acute alterations were reported in placental diffusing capacity

during maternal exercise when compared with pre-exercise control values. Despite the significant reduction in UBF during maternal exercise, uterine $\dot{V}O_2$ was maintained as a result of maintained oxygen hemoconcentration and increased uterine oxygen concentration. The authors suggested that acute maternal exercise of these intensities did not represent a major hypoxic or stressful event to the developing fetus (Lotgering *et al.*, 1983B).

The conclusions suggested by Lotgering *et al.* (1983A and 1983B) were in direct contrast to Emmanouilides *et al.* (1972), Longo *et al.* (1978) and Chandler and Bell (1981) who reported that maternal exercise may cause intrauterine growth retardation. Perhaps the familiarization of the animals in the studies of Lotgering *et al.* (1983A and 1983B) reduced the stress of an unfamiliar situation and apparatus.

The results of Lotgering *et al.* (1983A and 1983B) also support the findings of other researchers using catheterized pregnant ewes who found no alterations in fetal lambs as a result of acute maternal exercise (Orr *et al.*, 1972; Curet *et al.*,1976; Clapp, 1978; Clapp, 1980). However, it should be pointed out thât Orr *et al.* (1972) reported a non-significant increase in UBF during maternal exercise (no animal familiarization was reported). Curet *et al.* (1976) analysed UBF following maternal exercise and also reported no significant change. This latter group of authors 'trained' their pregnant animals for three weeks prior to measurement. The 'training' protocol, however, was a

treadmill speed of 3 miles/hour, 10% elevation to the point of exhaustion twice a week for the three weeks prior to experimentation. Clapp (1980) found a significant reduction in UBF in near-term pregnant ewes only with the onset of exhaustion (by acute sustained treadmill exercise). The pregnant animals were accustomized to a stationary treadmill for one to two hours with free availability of food and water (Clapp, 1980).

Errors in measured UBF may be an important variable during maternal exercise. For example, Lotgering *et al.* (1983A) listed four major problems. These included technical errors in the use of an electromagnetic flow probe, movement in the flow probe during maternal exercise resulting in vessel spasm, contraction, or stimulation of a sympathetic response, excessive catecholamine release before or during maternal exercise because of the stress of the treadmill and the new environment, and incorrect surgical techniques (such as denervation of uterine vessel sympathetic nerve supply).

The studies of maternal exercise in pregnant Pygmy goats should not go without mention. Dhindsa *et al.* (1978) reported that pregnant Pygmy goats, accustomed to standing on a treadmill and walking at 1.5 miles/hour up a 10° grade for ten minutes, one to two times per week, produced significantly smaller twin birth weight values than matched controls. The singleton births of exercised mothers were of normal weight. However, before each study session the pregnant goats were kept from food for 20 hours but were

allowed free access to water. Since maternal nutrition is an important element in fetal growth and development (Young, 1976), perhaps the 20 hour food deprivation in these pregnant goats in combination with maternal exercise may have played an important factor in fetal outcome, especially in multiple pregnancies.

Hohimer *et al.* (1984) measured UBF in exercised pregnant Pygmy goats, and in addition, these authors separated UBF into myoendometrial and cotyledonary (placental) components of uterine vasculature. Pregnant Pygmy goats (of approximately 80 days gestation) were taught to walk on a treadmill at a speed of 0.5 miles/hour for 10 minutes, three times/week. The speed was gradually increased to between 1.5 and 2.0 miles/hour up a grade of 10° or 15°, three times/week. This type of exercise did not affect birth weight values when compared to controls, even in twin births. However, uterine artery blood flow was decreased by 32% from control values and total uterine blood flow decreased by 18%; cotyledonary (placental) blood flow by 8%, while myoendometrial blood flow dropped by 52%. The authors concluded that the nonplacental portions of the uterus in pregnant Pygmy goats showed significant alterations in blood flow as a result of acute maternal exercise. This redistribution of blood flow from the myoendometrium to the placental portions would agree with Curet *et al.* (1976) who also reported the same effect as a result of maternal exercise in pregnant sheep.

In contrast to the report of Dhindsa *et al.* (1978), Hohimer *et al.* (1984), also from the same laboratory, reported no significant reduction in the birth weight values of twin kids born to goats of their study. Hohimer *et al.* (1984) based this difference on the way in which the goats were 'trained' to exercise. In the previous experiment adversive shock treatment was given to any animal that stopped exercising, whilst in the latter study the goats were 'trained' to exercise by rewarding the animal either with food or by petting when the pregnant goats performed well (Hohimer *et al.*, 1984).

PREGNANCY AND EXERCISE IN HUMANS:

The normal alterations in mammalian pregnancy that occur such as an increase in maternal blood volume, cardiac stroke volume and cardiac output and redistribution of organ blood flow are more pronounced in human pregnancies than in other mammals (NICHD Summary Report, 1982). Maternal hemodynamics in the human are uniquely sensitive to alterations in body position especially towards the later stages of gestation (Ueland *et al.*, 1969; NICHD Summary Report, 1982). In addition, normal maternal resting heart rates have been shown to increase approximately 10 to 15 beats/minute above non-pregnant values (Artal *et al.*, 1981), while in the pregnant rat, for example, no significant differences were found in resting heart rate values between paired pregnant and non-pregnant rats (Atherton *et al.*, 1982). The upright human posture and the pooling of venous

blood in the lower extremities (Metcalfe *et al.*, 1981) may cause the heart of the pregnant woman to work harder to compensate for this pooled blood and hence the elevated heart rate.

Not only do problems exist in comparing quadruped data to human data but also the following problems are found in the literature relating to human data alone:

1. The training state or preconditioned status of the exercising pregnant woman usually is not mentioned.

2. The assessment of exercise effects occur during different gestational periods, usually the third trimester.

3. The nutritional aspect of pregnant women cannot be controlled.

4. The position of the exercising woman must be taken into account. Supine positions are found to cause occlusion in the inferior vena cava by the pregnant uterus (Metcalfe *et al.*, 1981).

5. Weight-bearing exercise (such as treadmill running) cannot be compared to non-weight-bearing exercise (such as swimming and/or bicycle ergometer exercise) (Knuttgen and Emerson, 1978).

6. The intensity of the maternal exercise must be

taken into account.

7. Subject criteria such as weight and age must also be reported.

8. The differences between the effects of voluntary maternal exercise, such as recreational activities, and forced maternal activity such as that experienced by women who must seek employment outside the home, must be considered.

Acute Responses to Exercise in the Pregnant Human:

In 1970, Guzman and Caplan reported the monthly cardiorespiratory response to standardized mild and moderate bicycle ergometer exercise in 8 pregnant women from the first trimester until term and 3 months postpartum. The parameters measured included; maternal heart rate (HR), respiratory frequency (f), $\dot{V}O_2$, and cardiac output (CO) at each of the exercise levels; 150, 250 and 350 kg-m/min. Ten minutes of rest were allowed following each test. The women had a mean age of 24.2 years (range=16 to 33 years). The results showed that as pregnancy advanced, the resting ventilation increased non-proportionately with the amount of oxygen consumed and thus a resulting decrease in alveolar pCO_2 occurred. During exercise this same occurrence was replicated with the resultant decline in alveolar pCO_2. There was a progressive rise in HR throughout gestation which was most noted at the lowest workload. At increasing levels of work, HR was significantly higher than postpartal

values. There was no decline in resting CO near term perhaps because the subjects were measured in the sitting position rather than in supine recumbent. There was an increase in exercise CO per unit increase in oxygen uptake and this was proportionately similar in all stages of gestation which compared well to control measurements. This indicated good myocardial reserve. However, the pregnant women reached maximum CO at a lower level of exercise than when non-pregnant. The increase in CO during exercise was attributed to an increase in HR and SV, with HR contributing more during late gestation, particularly at the lower exercise workloads. The authors concluded that whatever the mechanism of the "hyperkinetic" aspect of gestation, it appeared to be well established before the 20th week, did not change with the size of the uterus or conceptus, and was sustained until the time of birth. The rate of increase in ventilation and in CO with increasing work done were similar throughout gestation as in the state of postpartum which may imply that the physiological response to exercise is the same in both conditions. However, in the pregnant state, women reach their maximal work tolerance at a lower work level than in the non-pregnant (postpartal) state (Guzman and Caplan, 1970).

Ueland *et al.*(1973) suggested that standard exercise on a bicycle ergometer costs more, with regard to oxygen consumption, at term than it does in early pregnancy or postpartum but this difference is not significant. The

greater response of the maternal heart during exercise (sitting) and the increased mechanical effort of breathing, to a standard workload evokes a greater CO than does the same workload postpartally. The larger CO supplies a high mean oxygen tension in the peripheral capillaries expecially during early pregnancy at rest and during this type of standard work. The kidneys and uterus are thought to be hyperemic during early pregnancy and this adjustment persists during exercise in the sitting position. Thus, these tissues have their oxygen needs provided at a relatively high pO_2.

Pernoll *et al*. (1975) measured oxygen consumption in 12 normal pregnant women, at rest, sitting on a bicycle ergometer and during standard steady-state exercise. The subjects ranged in age from 25 to 34 years, with a mean weight of 60.6 kg (range 53.2 to 69.5 kg). The workload was 50-Watts, pedalling rates of 40-50 rpm, with a fixed workload of 306 kpm/min. The duration of exercise was 6 minutes. The results showed a progressive increase in resting $\dot{V}O_2$ as pregnancy advanced, reaching 33% above non-pregnant levels (12 to 14 weeks postpartum) by 39 to 42 weeks gestation. $\dot{V}O_2$ during standard steady-state exercise also increased progressively as pregnancy advanced reaching a mean of 15% above non-pregnant values (12 to 14 weeks postpartum) at 39 to 42 weeks of gestation. Using paired t-test values, a significantly greater oxygen debt (approximately 14% larger) was found at 30 to 42 weeks of

pregnancy compared to 12 to 14 weeks postpartum. The authors stated that a small portion (about 20%) of the increased oxygen cost of exercise during pregnancy may be due to the increased work of the respiratory muscles associated with hyperventilation. CO also increases more during standard exercise in the pregnant woman, thus elevated myocardial $\dot{V}O_2$ may add to the rise in oxygen debt of maternal exercise during pregnancy. However, the augmented $\dot{V}O_2$ during exercise and the oxygen debt incurred in late pregnancy when compared to postpartal values, appeared to be substantially larger than could be explained by the increased amount of respiratory and myocardial work. They concluded that the efficiency of performing mild maternal muscular exercise decreased in humans during pregnancy (Pernoll *et al.*, 1975).

Artal *et al.* (1981) exercised pregnant women for 15 minutes on a motorized treadmill at a constant speed of 2 mph. This type of activity was considered to be "mild" exercise estimating an oxygen consumption of under 0.5 l/min. Maternal average heart rate increased to 104±2.7 beats/min. Dressendorfer and Goodwin (1980) had their pregnant subjects pedal a bicycle ergometer while sitting in a semi-recumbent position, progressively increasing the workload by 150 kg-m/min every 2 to 3 minutes until maternal heart rate approached a pre-determined endpoint of 150 beats/min. Oxygen consumption for the exercising pregnant women on the ergometer increased from a resting value of 0.27 l/min to 1.85 l/min at a maternal heart rate of 146

beats/min. This was considered to be aerobic exercise of approximately 80% of maximum. Maternal systolic blood pressure values increased substantially in both groups of exercising pregnant women.

It is interesting to note that in both groups of exercising women, whether they were engaged in weight-bearing or non-weight bearing activities, oxygen consumption increased proportionately to the amount of work done as determined by oxygen consumption at the working maternal heart rate. Resting maternal heart rate was approximately 12 beats/min lower in the more "fit" group during the same gestational time period.

The classic study by Morris *et al.* (1956) should not go without mention. The authors measured UBF before, during and after exercise in 21 normal pregnant and 19 pre-eclamptic pregnant women. The women exercised on an "exercycle" devised for bed use and each woman pedalled to maintain a speed of 10 mph for 4-5 minutes. They then stopped, and diffusable sodium ion injections were made into uterine and leg sites. They continued cycling for 6-9 minutes more, reaching an average of 450 revolutions in 10-16 minutes of total work done. There was no difference in the amount of work done between the normal and hypertensive groups. The results showed that blood flow to the thigh muscle during exercise almost doubled from resting values with a decrease in UBF. On cessation of exercise the blood flows were reversed. The initial restoration of UBF was so dramatic

that it was termed a "flush-back" of blood to the uterus. These researchers concluded that during normal pregnancy resting blood flow to the abdominal viscera is in excess of required oxygen so that during exercise a considerable amount of the blood supply can be shunted to maternal working muscles without detriment. In a normal pregnancy the ability of the fetus to withstand blood flow fluctuations from maternal activity depends on the amount of reserve and on a functionally efficient placenta. The "flush-back" effect was found to be greater in the pre-eclamptic women which may suggest that uterine hypoxia may be greater than in the normal pregnant woman (Morris *et al.*, 1956). However, the women in Morris *et al.* study (1956) participated in the exercise while in bed in the supine recumbent position and since supine position during pregnancy has been shown to occlude the IVC and the abdominal aorta (Metcalfe *et al.*, 1981), it raises the question of whether the values reported are not actually artifact resulting from the maternal position during exercise.

Physical Training During Pregnancy In Humans:

Erkkola (1976A) examined the influence of physical training during pregnancy on physical work capacity (PWC) and circulatory parameters in a group of 6 healthy pregnant women. The subjects were primigravidae between 20 and 26 years of age. The pregnant women were divided into 2 groups. The training group began a training program in the 10th to 14th week of pregnancy and continued until term. These women

were instructed to perform one hour of strenuous exercise three times per week throughout pregnancy. The women controlled the intensity of the exercise session by monitoring HR to 140 beats/min several times during the hour. During the first two trimesters all exercise types were recommended but during the last trimester bumping or compressing exercises which compressed the uterus were not allowed. All of the women in the training group exercised over 60 hours total training time and about 50% of them exceeded 80 hours. The control group did not participate in any extra activities. In the 38th week of gestation all the women had a preliminary 12 minute work bout on a bicycle ergometer. The test ended when maximum voluntary fatigue occurred. The results showed that the pregnant training group improved their "physical fitness" by 27% which was significantly higher than in the control group. The PWC in the training group improved about 10% during pregnancy and at 2 weeks before the end of gestation PWC was at the same level as in non-pregnant women of the same age. At term all HR and blood pressure readings were lower in the training group compared to the control group but these differences were not significant. Erkkola (1976A) concluded that training had little influence on HR and blood pressure parameters during pregnancy. No disturbances in pregnancy were noted as a result of the submaximal exertion test on healthy women in various aspects of gestation although the effect on the fetus were not measured directly (Erkkola,

1976A).

In another study by Erkkola (1976B), he determined the correlation between maternal PWC and the course of pregnancy, labour and birth outcome. The 149 pregnant women were between 20 and 26 years of age. PWC was analyzed in the same manner as his previous experiment (Erkkola, 1976A). All the women were examined on the 38th week of gestation. The results showed that pregnant women with a PWC above the mean value for non-pregnant women had an "almost" significantly shorter labour time, when it was spontaneous. There was almost a significant difference between infants weighing over 3.5 kg (7.7 lb) in this group of women than in the group with a PWC value below that for non-pregnant women. The more "fit" group also had heavier placental weights (without umbilical cord and membranes). As mentioned previously Erkkola (1976B) presumed that a high maternal PWC leads to a heavier placenta with a better circulation and gaseous exchange which is therefore beneficial for fetal well-being.

Dressendorfer and Goodlin (1980), monitored maternal heart rate (HR) in the 5 pregnant physically fit women (between 32 and 39 weeks gestation) and fetal heart rate (FHR) responses <u>during</u> maternal exercise on a bicycle ergometer were also measured. These pregnant women participated in a moderate endurance training program consisting of lap swimming 30 to 45 minutes at least three times/week during pregnancy. The exercise stress test which

monitored fetal and maternal HR was progressively increased from 150 kg-m/min every 2-3 minutes until each subject completed a workload of 150, 300, 450 and 600 kg-m/min without volitional fatigue. The predetermined end point for maternal HR was 150 beats/minute. The measurements of maternal and fetal HR were obtained during exercise in the last minute of each workload. The average fetal base-line HR before exercise was 142 beats/minute (range=135 to 152). FHR recorded at peak exercise averaged 149±5 beats/minute. The results indicated that the HR's in both mother and fetus increased linearly with greater production of aerobic energy. However, FHR increased by only 7 beats/minute above the base-line pre-exercise level while maternal HR rose by 70 beats/minute. FHR increased approximately one beat/minute to every 10 beats/minute increase in maternal HR during graded submaximal exercise. The author's suggestions to the possible causes of this slight but uniform augmentation in FHR were an elevated circulating catecholamine level in the exercising women, perhaps the increase in fetal movements that were also noted during exercise, and an increase in uterine contractions. Since extreme beat to beat variability in FHR at rest and during exercise occurred and was found to range between 5 to 20 beats/minute and a cyclic variability in FHR of 10 to 20 beats/minute was observed in all subjects, the authors concluded that the findings portrayed a normal FHR response to dynamic exercise of a submaximal workload and the aerobic exercise which increased maternal

HR to about 80% of max $\dot{V}O_2$ did not elicit changes leading to sustained fetal bradycardia or tachycardia.

Collings *et al.* (1981) trained 12 pregnant women at 65 to 70% of their max $\dot{V}O_2$ for three 30-minute sessions per week on a bicycle ergometer throughout the second and third trimester. Acute fetal responses were assessed by auscultation of FHR before, during and after the exercise sessions. Average FHR 10 minutes (average=147 beats/minute) and 20 minutes (average=148 beats/minute) into the exercise session and 5 minutes after the exercise bout (average=147 beats/minute) were significantly greater than pre-exercise FHR values of 143 beats/minute ($p<0.01$). Long term fetal effects from maternal training were determined by the comparison of birth outcome (birth weight, length, placental weight, 1 to 5 minute Apgar scores and gestational age) between the training group and a control group (who did not participate in any regular exercise program). There were no significant differences between the training group and the control group with regards to birth outcome or labour duration. The authors concluded that FHR increased slightly during maternal exercise but still remained within normal limits. Regular exercise of this type did not influence length of labour or birth outcome (Collings *et al.* 1981).

Dale *et al.* (1982) studied the effects of running during pregnancy involving both retrospective and longitudinal neonatal evaluation of 33 pregnant runners and 11 controls (pregnant non-runners). The retrospective sample

included women who experienced pregnancy in the past 5 years and ran during the duration of that pregnancy. These 21 women were between 24 to 35 years of age (average=30 years) with a prepregnancy weight of 50 kg (110 lb.). All of the 21 women had run prior to pregnancy with a mean prepregnancy distance/week of 18.7 miles. The average distance run in the first trimester of pregnancy was 14.2 miles/week; 10.9 miles/week in the second trimester and 6.6 miles/week during the last. The average prepregnant running speed was 8.5 min/mile which increased to 10.25 min/mile during gestation. Infant birth weights were from 2.0 kg (4 lb 7 oz) to 4.36 kg (9 lb 9 oz) with a mean birth weight of 3.4 kg (7.5 lb.). All infants except 2, were born at term.

This longitudinal study (Dale *et al.*, 1982) monitored simultaneously maternal and fetal HR patterns before, during and after exercise. Twelve pregnant runners were assessed in this manner while engaging in treadmill running. The subjects were monitored in the left lateral position for 10 minutes prior to treadmill running. The exercise protocol began at 2 miles/hour with an incline of 3°. After 2 minutes, the speed increased to 4 miles/hour with an incline of 5%. Three minutes later the speed was kept constant while the incline was increased to 10%. From standardized charts maternal submaximal HR was determined to be approximately 80% predicted max $\dot{V}O_2$. Upon completion of the exercise test, the subjects returned to the left lateral position for another 15 minutes. Eleven pregnant controls were matched

with the group of 12 pregnant runners. The mean age was 28 years and the mean prepregnancy weight in the running group was 51.4 kg (113 lb) and for the control group the average was 52.7 kg (115.9 lb). This running group averaged 13 miles/week for the first trimester; 12.9 miles/week for the second and 9.2 for the last. Training speeds decreased from 9 min/mile to 12 min/mile during gestation. Mean infant birth weights were 3.4 kg (7.45 lb) and 3.5 kg (7.6 lb), respectively. The results of FHR monitoring in 3 pregnant women showed that initially during the exercise session there was a temporary decrease to 90-115 beats/min in FHR for 2 to 3 min., (compared to baseline levels of 125-140 beats/min), with a recovery to the normal range (120 to 160 beats/min) after the 3 to 3.5 min mark and before submaximal cardiac level was achieved.

The results from Dale *et al.* (1982) showed no significant differences between the women who ran during pregnancy and the non-runner controls regarding labour length and delivery, and incidence of complications. There was, however, a "suggestive trend" in the training group, of failure to progress during labour which resulted in an increased rate of cesarean section and more frequent major obstetric complications were found in the running group. Birth outcome showed that average infant birth weights were almost similar in both groups. However, there were more neonatal complications reported in the control group. The fetal bradycardia reported during the exercise test should

not be ignored. It was fortunate that the bradycardia was temporary and that recovery to normal patterns occurred while exercise was still in progress. The cause of the depressed FHR was difficult to explain but it does not appear to be due to the generation of metabolic acids (ie. lactic acid) because time of occurence was insufficient. The authors concluded that these results suggested that exercise stimulus of this type did not seriously compromise the function of the uteroplacental system (Dale *et al.*, 1982).

By comparison of the FHR base-line values found in the study by Dressendorfer and Goodlin (1980) (135 to 152 beats/min) to base-line values (125-140 beats/min) found for Dale *et al.* (1982), there definitely is a large variation between these reports even though both are within the normal FHR range (120 to 160 beats/min) (Dressendorfer and Goodlin, 1980). Both groups of pregnant women were defined as physically fit; in the first study the pregnant women swam (weight-supported) while in the second study the pregnant women jogged (weight-bearing). The exercise stress test performed on both groups of women elicited a work load of approximately 80% $\dot{V}O_2$ but the first study was performed on a bicycle ergometer (again weight-supported) and the second was performed on a treadmill (again including maternal weight). Both exercise tests were performed during the last trimester of pregnancy. The slight temporary decline in FHR reported in the treadmill group (90 to 115 beats/min) for 2 to 3 minutes during exercise is interesting when this did

not occur in the weight-supported group; in fact a slight increase in FHR occurred. This difference is difficult to explain but perhaps the small number of pregnant women monitored in both groups (n=5 and n=3, respectively) and the inaccuracies found in recording FHR especially during maternal movements can add some insight. It is interesting to note that Collings *et al*. (1981) also trained pregnant women on a bicycle ergometer and reported FHR values comparable to Dressendorfer and Goodlin (1980). The difference in baseline levels found could be attributed to the different maternal positions used for base-line monitoring; ie. Dressendorfer and Goodlin (1980) and Collings *et al*. (1981) measured the pregnant women while seated on a bicycle ergometer and Dale *et al*. (1982) monitored FHR while the pregnant women were in the left lateral position. As reported previously, maternal position can influence maternal resting cardiovascular measurements because of its effect on the inferior vena cava. Perhaps weight-bearing exercise in the human can somehow affect FHR response even though it appeared to be transitory.

Collings *et al*. (1983) assessed the effects of training on maternal and fetal responses. Twenty pregnant women participated in the study. Twelve of the twenty women participated in a supervised exercise program based on each woman's max $\dot{V}O_2$. The resultant training intensity for the exercising women was 65% to 70% max $\dot{V}O_2$ and the training heart rate average 152 beats/minute. The women exercised

three times/week for about 13.4 weeks and each exercise session began with 10 minutes of flexibility warm-up exercises, followed by 10 minutes of light pedalling on a bicycle ergometer. Each woman then pedalled at her prescribed training intensity for 25 minutes. Fetal and maternal HR were monitored. The results showed an 8% improvement in functional aerobic capacity of the training women when compared to the control women who did not exercise during pregnancy. The results also suggested that maternal exercise of this level did not affect fetal growth, as indicated by no difference in birth weight, birth length, and placental weight values when compared to controls. A small significant increase was found in FHR values during maternal exercise of this intensity level (from 144 beats/minute to 148 beats/minute) although these values were within normal range. No beneficial or detrimental effects were seen on labour duration as a result of the maternal exercise (Collings *et al.*, 1983).

There is one other factor that must be considered when assessing exercise and pregnancy in the human and that is the effects of working outside the home during pregnancy on fetal outcome. Naeye and Peters (1982) analysed 7,722 women who were placed in one of three work categories: they did not work outside the home; had employment outside the home that required sitting most of the time; or had employment outside the home that required standing most of the time. The study reported that women who held employment outside

the home had lower birth weight values than women who remained at home during the last trimester of pregnancy. The smaller birth weight values were most severe when the employed women had stand-up jobs, continued employment until near the end of gestation, were hypertensive or had other children to care for when they returned home from work (Naeye and Peters, 1982).

The results of Naeye and Peters (1982) demonstrate one more factor that cannot be controlled when assessing the effects of maternal activity on fetal outcome, that of working outside the home. It is interesting to note that of the human data presented, neither acute maternal exercise nor pregnant women who trained during pregnancy, showed any detrimental effects on fetal outcome. However, fetal growth retardation was found in women who were required to work during pregnancy. The data collected on women engaged in maternal exercise were volunteers, while the pregnant women working outside the home may be 'forced' to do so. Perhaps in human research, the added emotional and mental stress of employment complicates the actual assessment of 'physical work' that occurs outside the home.

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APPENDIX A

PROGRESSIVE RUNNING BEHAVIOUR CHART

(Example of first week in MILD experiment)

PROGRESSIVE RUNNING BEHAVIOUR CHART

(Example of first week in MILD experiment)

Rat ID	Day	Treadmill Speed (m/min)	Incline (degrees)	Time (minutes)	Comments
	1	15	10	15	all rats fine
	2	15	10	20	
M02					trouble running
M07					problem running
	3	15	10	30	
M05					diarrhea
M07					squeeky
M09					slight diarrhea
M08					good runner
	4	15	10	40	
M07					diarrhea
M05					diarrhea
others					accustomed to treadmill
	5	20	10	50	
M03					good runner
M02					intermittent runner
M07					bad runner
	6				REST
	7				REST

APPENDIX B
PREGNANT RUNNING BEHAVIOUR CHART
(Example of first week in SEV experiment)

PREGNANT RUNNING BEHAVIOUR CHART
(Example of first week in SEV experiment)

Rat ID	Day	Treadmill Speed (m/min)	Incline (degrees)	Time (minutes)	Comments
	1	30	10	120	
PR302					cut paw, ran poorly
PR301					both paws cut and swollen
	2	30	10	120	
PR302					day off
PR301					day off
	3	30	10	120	
PR309				90	swollen paws, ran poorly
PR3010					cut paw
PR301					okay
PR302					okay
	4	30	10	120	all okay
	5	30	10	120	
PR3010					swollen paw
PR309					cut paw
	6				REST
	7				REST

APPENDIX C

METHODOLOGY FOR ACTOMYOSIN ATPase

SOLUTIONS

1. No solution 1 - fixative was not used.

2. Rinse solution (18 mM $CaCl_2$ in 100mM tris) (hydroxymethly) aminomenthane (Tris), pH 7.8.

Tris (MW 121)	12.1 g
$CaCl_2$ (0.18M)	100 ml
Distilled water	900 ml

3. Alkaline preincubation (18mM $CaCl_2$ in 100 mM buffer, pH 10.4)

Sigma No 221 buffer (1.5 M)	3.35 ml
$CaCl_2$ (0.18 M)	5 ml
Distilled water	40 ml

4. Incubation solution (2.7mM ATP, 50 mM KCL, 18 mM $CaCl_2$ in 100 mM buffer, pH 9.4).

Sigma No 221 buffer (1.6 M)	3.35 ml
$CaCl_2$ (0.18 M)	5 ml
KCL (MW 75)	0.185 mg
ATP disodium (MW 551.2)	0.076 mg
Distilled water	40 ml

5. Wash solution (1% $CaCl_2$, w/v).

$CaCl_2$ (MW 147)	10 g
H_2O	1000 ml

6. Cobalt chloride solution (2% w/v).

$CoCl_2$(MW 238)	1 g

H_2O	50 ml

7. Alkaline washing solution (100 mM buffer, pH 9.4).

Sigma No 211 buffer (1.5 M)	13.4 ml
H_2O	160 ml

8. Ammonium sulfide solution (1% w/v).

Ammonium sulfide (light)	0.5 ml
H_2O	50 ml

Sigma (1.5 M solution)

Sigma (MW 89.1)	14.4 ml
Distilled water	100 ml

Bring 14.4 ml up to 100 ml with distilled water.

METHOD

1. Dry frozen sections for 90 min at room temperature.

2. Rinse slide in Solution 2 for 1 min, with agitation and drain.

3. Preincubate in Solution 3 for 20 min (in fridge, no agitation).

4. Rinse slides in Solution 2 (two changes, 1 min each) and drain excess solution.

5. Incubate for 15 min in Solution 4 at 37°C. (The Solution 4 is filtered into staining jar that is prewarmed to 60°C. This rapidly warms the solution

to about 37°C).

6. Wash in three 30 sec changes of Solution 5 and drain excess solution.

7. Place in solution 6 for 5 min (oven).

8. Wash in four 30 sec changes of Solution 7 and drain excess solution.

9. Place in Solution 8 for 3 min (oven).

10. Wash in running tap water for 3-5 min, dehydrate in graded ethanol, clear in xylene, and mount in Permount.

APPENDIX D

TABLE 9. CUMULATIVE AVERAGE WEIGHT GAIN DURING 21 DAY GESTATION PERIOD FOR GROUPS PR & PC (MILD, MODERATE, AND SEVERE) AND C, NPR-SEV

TABLE 10. PROPORTIONS OF MATERNAL WEIGHT VALUES AND FETAL OUTCOME VALUES FOR MILD, MOD, SEV

Table 9. CUMULATIVE AVERAGE WEIGHT GAIN (EXPRESSED IN (g)) DURING 21 DAY GESTATION PERIOD FOR GROUPS PR & PC (MILD, MODERATE, SEVERE) and C, NPR-SEV

GROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
C	0.6	3.5	6.0	7.0	8.8	9.8	13.4	13.8	14.5	16.0	20.4	19.4	25.0	26.4	28.4	29.5	31.0	31.6	35.8	35.6	36.2
	(5.5)	(5.1)	(5.0)	(4.6)	(8.4)	(6.3)	(6.3)	(4.5)	(4.3)	(4.4)	(4.2)	(4.9)	(9.3)	(7.6)	(5.2)	(5.6)	(6.0)	(6.6)	(6.1)	(8.2)	(7.8)
PC	4.0	6.8	9.5	10.0	16.7	20.6	22.4	25.4	31.3	36.0	39.3	46.7	53.4	66.4	82.0	93.8	105.0	111.0	115.0	132.0	142.0
MILD	(3.1)	(7.0)	(3.5)	(6.9)	(10.4)	(7.6)	(4.8)	(5.8)	(6.3)	(5.7)	(6.6)	(7.6)	(6.5)	(8.7)	(5.7)	(4.8)	(1.4)	(5.0)	(8.0)	(11.0)	(14.0)
PR	1.8	2.7	4.0	8.3	12.5	15.0	15.8	19.5	28.0	30.3	33.3	35.0	42.5	54.5	63.8	74.3	87.7	92.5	99.3	107.0	115.0
MILD	(2.2)	(2.5)	(1.4)	(6.5)	(3.5)	(4.0)	(4.3)	(6.7)	(5.0)	(6.8)	(1.5)	(6.6)	(10.6)	(4.2)	(6.5)	(15.0)	(15.6)	(13.4)	(4.6)	(8.0)	(17.0)
PC	7.2	9.7	12.4	17.3	20.1	21.8	26.1	29.4	32.1	37.8	41.7	46.4	51.5	58.2	65.8	76.0	89.2	102.1	116.3	133.0	144.3
MOD	(4.6)	(4.5)	(4.3)	(6.9)	(5.8)	(6.4)	(5.2)	(5.5)	(3.9)	(4.9)	(4.2)	(3.9)	(6.0)	(5.3)	(5.7)	(6.8)	(5.8)	(7.3)	(9.1)	(12.0)	(13.1)
PR	3.2	5.5	8.2	9.3	14.0	15.4	17.7	17.7	18.7	24.5	29.6	32.0	37.0	39.0	45.3	53.6	63.4	79.1	91.2	99.3	110.4
MOD	(3.1)	(4.3)	(3.8)	(1.6)	(6.6)	(5.2)	(4.2)	(6.2)	(6.8)	(7.5)	(6.6)	(7.0)	(7.5)	(7.5)	(6.5)	(7.4)	(9.6)	(9.3)	(10.1)	(19.1)	(22.9)
PC	7.4	11.3	13.8	17.9	22.8	25.4	29.3	32.3	37.3	40.4	46.8	52.0	55.3	59.1	65.8	75.9	87.8	101.1	114.5	125.9	140.1
SEV	(4.0)	(1.0)	(4.1)	(6.2)	(7.2)	(5.9)	(6.9	(8.3)	(8.5)	(11.7)	(11.0)	(13.6)	(12.1)	(14.4)	(14.8)	(10.9)	(14.5)	(13.3)	(18.5)	(19.7)	(18.9)
PR	3.3	8.3	9.3	11.8	14.9	17.0	20.6	21.1	24.8	27.8	29.8	33.3	36.9	40.6	46.5	53.3	64.4	73.9	83.6	90.5	102.0
SEV	(4.5)	(4.7)	(5.2)	(4.8)	(5.1)	(6.1)	(3.7)	(5.6)	(8.1)	(5.1)	(6.6)	(7.2)	(6.5)	(7.8)	(6.7)	(5.3)	(5.6)	(5.9)	(6.0)	(9.0)	(10.2)
NPR	1.1	3.8	5.6	4.6	4.0	5.4	6.6	7.6	6.5	5.6	7.9	7.3	8.6	9.6	9.9	11.0	13.1	15.4	16.3	17.8	15.0
SEV	(5.1)	(4.8)	(7.0)	(7.0)	(7.1)	(6.0)	(6.3)	(5.8)	(6.8)	(5.9)	(5.1)	(4.9)	(3.5)	(3.8)	(6.1)	(5.9)	(5.2)	(5.3)	(3.7)	(4.1)	(4.8)

Table 10. PROPORTIONS OF MATERNAL WEIGHT VALUES AND FETAL OUTCOME VALUES FOR MILD, MOD, SEV•

GROUP	TOTAL LITTER WEIGHT: MATERNAL WEIGHT %	PUP WEIGHT: MATERNAL WEIGHT %
PC-MILD	24.6	1.9
PR-MILD	20.6	2.1
PC-MOD	21.8	1.8
PR-MOD	21.4	2.0
PC-SEV	20.6	1.8
PR-SEV	19.8	2.1

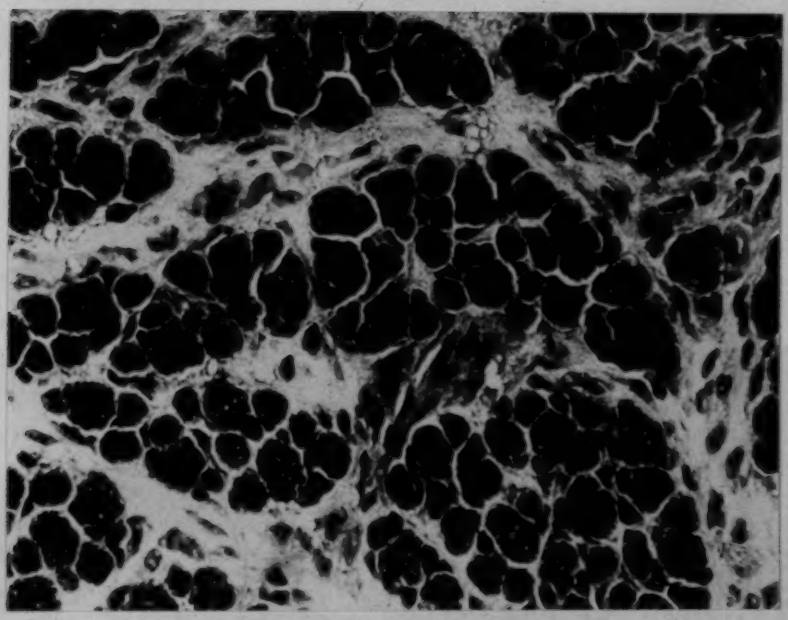
* These ratios were calculated using the average weight values for each group.

APPENDIX E

PHOTOGRAPHIC PLATES OF NEWBORN GASTROCNEMIUS, STERNOMASTOID AND DIAPHRAGM MUSCLE

PLATE 1A - TYPICAL NEWBORN SKELETAL
MUSCLE: GASTROCNEMIUS.
MAGNIFICATION X 415.
MASSON'S TRICHROME STAIN.

PLATE 1B - TYPICAL NEWBORN SKELETAL
MUSCLE: STERNOMASTOID.
MAGNIFICATION X 525.
MASSON'S TRICHROME STAIN.



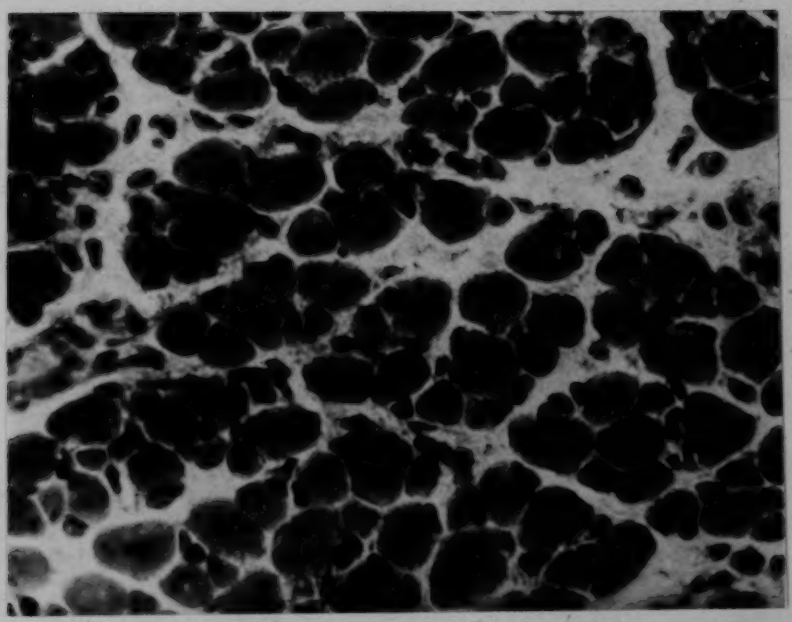
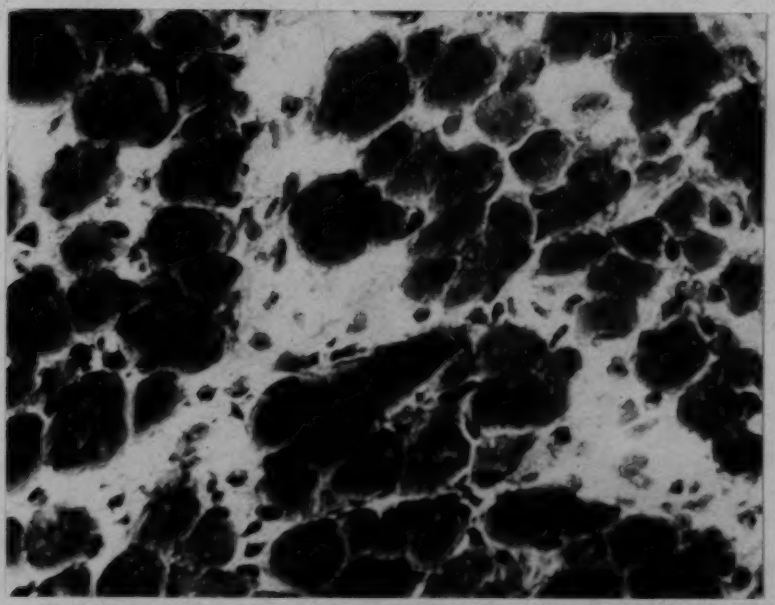
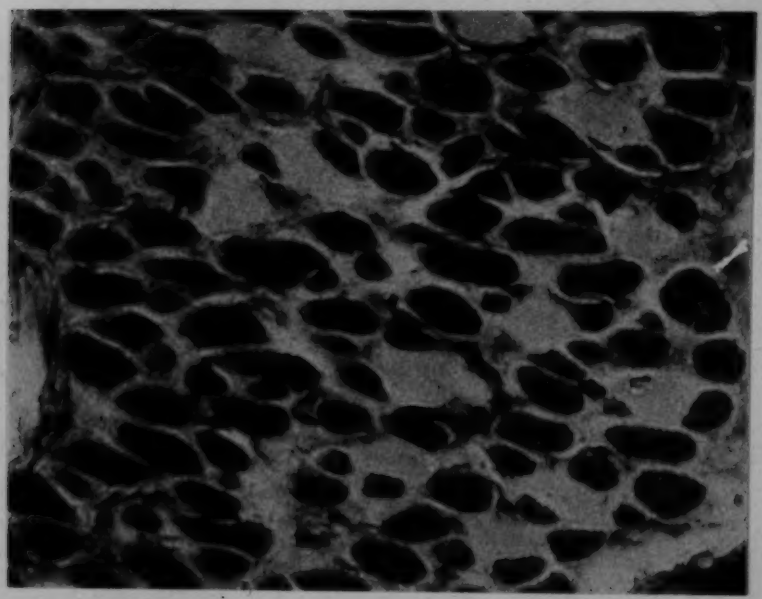


PLATE 2A - NEWBORN DIAPHRAGM MUSCLE.
MAGNIFICATION X 525.
MASSON'S TRICHROME STAIN.

PLATE 2B - NEWBORN DIAPHRAGM MUSCLE.
MAGNIFICATION X 400.
MYOSIN ATPase STAIN.
(PREINCUBATED AT pH 10.4).





APPENDIX F

SUMMARY OF PAIRED T-TEST RESULTS

SUMMARY OF PAIRED T-TEST RESULTS

1. Weights of Paired Female Rats on Day of Conception

   a. PC-MILD and PR-MILD
      $t(3)=2.23$
      $p=0.11$

   a. PC-MOD and PR-MOD
      $t(9)=0.61$
      $p=0.56$

   b. PC-SEV and PR-SEV
      $t(7)=1.90$
      $p=0.10$

2. The Number of Days Prior to Conception

   a. PC-MILD and PR-MILD
      $t(3)=2.10$
      $p=0.13$

   b. PC-MOD and PR-MOD
      $t(9)=1.34$
      $p=0.21$

   c. PC-SEV and PR-SEV
      $t(7)=0.94$
      $p=0.07$

3. Last Recorded Pregnancy Weights Before Giving Birth

   a. PC-MILD and PR-MILD
      $t(3)=2.71$
      $p=0.07$

   b. PC-MOD and PR-MOD
      $t(9)=5.05$
      $p=0.007$

   c. PC-SEV and PR-SEV
      $t(7)=4.47$
      $p=0.003$

4. Postpartal Weights for the Pregnancy Groups Within 20 Hours After Giving Birth

   a. PC-MILD and PR-MILD
      $t(3)=0.33$
      $p=0.76$

   b. PC-MOD and PR-MOD
      $t(9)=2.24$
      $p=0.05$

   c. PC-SEV and PR-SEV
      $t(7)=3.19$
      $p=0.015$

5. Postparal Weight Gain for the Pregnant Group

   a. PC-MILD and PR-MILD
      $t(3)=1.45$
      $p=0.24$

   b. PC-MOD and PR-MOD
      $t(9)=3.18$
      $p=0.01$

   c. PC-SEV and PR-SEV
      $t(7)=2.49$
      $p=0.04$

6. Average Weights of Each Litter

   a. PC-MILD and PR-MILD
      $t(3)=0.33$
      $p=0.76$

   b. PC-MOD and PR-MOD
      $t(9)=0.12$
      $p=0.91$

   c. PC-SEV and PR-SEV
      $t(7)=0.43$
      $p=0.68$

7. Average Number of Neonates/Litter

   a. PC-MILD and PR-MILD
      $t(3)=1.40$
      $p=0.26$

   b. PC-MOD and PR-MOD
      $t(9)=1.08$
      $p=0.31$

   c. PC-SEV and PR-SEV
      $t(7)=1.06$
      $p=0.44$

8. Average Total Litter Weights

   a. PC-MILD and PR-MILD
      $t(3)=1.41$
      $p=0.25$

   b. PC-MOD and PR-MOD
      $t(9)=0.15$
      $p=0.15$

   c. PC-SEV and PR-SEV
      $t(7)=1.69$
      $p=0.14$

SUMMARY OF ANOVA RESULTS

1. Weights of Paired Female Rats on Day of Conception
   MSt=307.8 MSe=218.7
   F(5,39)=1.408 p=0.24

2. The Number of Days Prior to Conception
   MSt=12.3 MSe=16.6
   F(5,39)=0.74 p=0.60

3. Last Recorded Pregnancy Weights Before Giving Birth
   MSt=2747.8 MSe=285.7
   F(5,39)=9.616 p=0.00005
   Scheffé Results

   a. PC-MILD and PR-MILD
      p=0.358

   b. PC-MOD and PR-MOD
      p=0.005

   c. PC-SEV and PR-SEV
      p=0.004

4. Postpartal Weights for the Pregnancy Groups Within 20 Hours After Giving Birth
   MSt=1124.2 MSe=323.1
   F(5,39)=3.481 p=0.0107
   Scheffé Results

   a. PC-MILD and PR-MILD
      p=0.941

   b. PC-MOD and PR-MOD
      p=0.636

   c. PC-SEV and PR-SEV
      p=0.348

APPENDIX G
RAW DATA TABLES

Table 11. DAILY WEIGHTS DURING PREGNANCY OF GROUPS PC, PR AND PNRC, AND C FOR THE MILD EXPERIMENT (expressed in grams).

RAT ID	DAYS																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PC																						
MO2	235	235	245	250	260	265	268	270	275	275	270	275	288	295	300	305	320	330	340	365	378	390
MO7	255	255	262	265	268	270	278	280	282	286	288	290	294	305	310	310	323	329	335	350	360	380
MO8	225	230	235	238	240	240	245	248	250	250	255	260	268	270	281	285	290	300	325	338	350	380
MO5	220	220	225	225	225	228	230	230	240	245	245	250	260	265	270	270	300	310	315	344	353	365
MO9	200	200	205	210	212	215	215	225	230	225	230	235	238	240	245	255	260	278	285	298	310	330
PR																						
MO1	250	250	258	255	260	260	265	270	273	275	280	295	285	290	295	300	310	320	330	340	350	372
MO4	230	230	235	235	240	242	242	245	250	250	255	260	262	268	270	270	285	290	295	308	315	320
MO6	228	228	230	230	230	235	232	233	235	240	240	258	258	262	276	280	290	300	320	332	340	356
MO10	200	200	205	210	215	218	220	220	220	225	230	238	241	240	240	255	260	272	289	302	305	320
MO3*																						
PNRC																						
A	249	249	253	253	257	262	266	270	271	272	278	280	289	288	294	297	300	308	316	327	341	357
C	267	271	273	280	282	283	283	286	284	293	295	300	308	307	309	315	324	343	362	378	390	402
E	267	271	272	277	284	280	282	289	284	288	290	292	295	312	314	323	335	349	368	385	398	409
2	246	250	258	261	263	264	265	262	273	276	278	286	286	291	295	306	312	328	345	355	367	380
4	240	249	245	252	257	262	260	262	269	273	277	291	290	292	304	312	320	336	355	365	395	413
6	210	217	219	221	223	226	231	232	237	247	249	263	259	262	263	276	278	298	311	329	343	354
C																						
NR1	218	222	223	224	225	234	232	240	230	235	240	242	240	250	252	252	252	252	252	258	252	258
NR2	205	208	211	215	218	220	212	220	225	225	230	230	230	232	222	235	235	234	234	242	248	245
NR3	210	202	205	210	212	205	210	215	220	222	224	225	222	220	230	230	231	232	232	235	240	238
NR4	212	215	215	215	215	220	225	222	222	223	225	230	230	235	240	240	242	245	245	250	238	240
NR5	225	220	225	230	235	235	240	240	242	243	244	245	245	258	258	255	259	262	265	264	270	270

* - dropped from study because did not appear to be pregnant.

Table 12. NEONATAL DATA FOR MILD EXPERIMENT.

RAT ID	NUMBER PER LITTER	AVERAGE PUP WEIGHT (g)	TOTAL LITTER WEIGHT (g)
PC			
MO2	14	6.7 (0.4)	94.3
MO8	15	7.0 (0.4)	105.2
MO5	12	7.7 (0.4)	92.1
MO9	11	6.8 (0.6)	75.1
MO7	13	6.7 (0.4)	87.0
PR			
MO1	9	8.3 (0.4)	74.8
MO4	6	6.9 (0.5)	41.5
MO6	12	8.1 (0.5)	97.7
MO10	12	5.6 (0.3)	67.7

Table 13. DAILY WEIGHTS DURING PREGNANCY OF GROUPS PC AND PR FOR THE MOD EXPERIMENT (expressed in grams).

RAT ID	DAYS																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PC																						
1	214	216	223	230	232	235	238	240	244	250	255	259	262	268	276	282	296	311	320	337	351	371
2	237	240	252	245	247	250	252	257	258	262	270	276	281	287	287	295	306	321	338	348	365	369
3	222	230	230	231	237	241	243	250	251	253	252	261	270	274	280	291	293	307	324	342	367	380
4	225	227	233	234	235	237	233	242	250	255	262	264	261	263	275	283	301	311	324	331	347	354
5	237	251	254	260	262	265	265	267	274	273	280	287	286	290	296	304	313	324	340	350	366	380
6	212	223	225	227	236	235	237	239	242	248	252	254	257	264	272	276	287	295	305	323	333	350
7	240	242	243	247	247	252	255	260	268	271	271	275	288	291	302	308	318	335	348	367	387	390
8	221	229	233	238	244	247	246	250	256	258	261	261	270	283	283	295	310	317	333	351	369	379
9	220	230	234	236	240	245	249	250	251	256	261	266	269	268	274	279	285	303	309	322	334	343
10	237	250	255	257	260	256	257	268	269	272	280	281	285	293	303	309	315	332	346	357	375	392
PR																						
1	203	209	213	214	213	212	215	218	220	223	225	228	236	240	243	248	254	261	278	293	304	325
2	236	237	238	240	242	245	248	251	249	252	261	261	267	275	276	284	291	308	324	336	355	369
3	248	248	250	254	255	255	256	260	261	260	265	266	269	270	***	306	312	328	345	355	367	380
4	209	208	212	217	220	227	223	225	225	230	235	243	245	253	247	257	265	277	284	289	300	306
5	212	215	221	228	223	232	229	230	234	238	242	246	246	254	260	264	275	285	296	308	319	356
6	219	226	223	226	229	232	235	239	239	237	250	248	254	258	259	264	270	276	288	294	302	308
7	229	237	242	246	249	253	255	256	258	258	266	270	273	276	281	286	295	303	324	337	353	369
8	230	229	235	236	240	237	244	248	242	243	246	251	255	260	266	272	280	288	304	321	334	343
9	242	246	244	246	252	252	259	259	253	256	260,	265	265	274	279	277	292	300	310	329	336	340
10	196	200	205	208	206	212	212	211	217	225	225	230	230	236	231	240	250	267	279	290	307	319

Table 14. NEONATAL DATA FOR MOD EXPERIMENT.

RAT ID	NUMBER PER LITTER	AVERAGE PUP WEIGHT (g)	TOTAL LITTER WEIGHT (g)
PC			
1	15	6.7 (0.4)	94.4
2	12	7.1 (0.3)	85.4
3	13	7.2 (0.4)	93.8
4	10	7.3 (0.7)	72.9
5	11	6.6 (0.6)	72.5
6	13	5.9 (0.5)	76.6
7	12	7.4 (0.5)	88.7
8	12	7.4 (0.3)	88.6
9	11	5.7 (0.5)	62.1
10	13	5.6 (0.3)	72.6
PR			
1	12	6.9 (0.6)	82.6
2	15	6.8 (0.4)	101.4
3	6	7.4 (0.4)	44.2
4	9	7.1 (0.3)	63.9
5	13	5.9 (0.5)	77.2
6	6	7.5 (0.3)	45.1
7	14	5.8 (0.4)	81.5
8	13	5.8 (0.3)	75.9
9	9	6.7 (0.7)	61.8
10	12	6.6 (0.4)	78.6

Table 15. DAILY WEIGHTS DURING PREGNANCY OF GROUPS PC AND PR, AND NPR FOR THE SEV EXPERIMENT (expressed in grams).

RAT ID	DAYS																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PC																						
302	251	261	262	267	271	279	276	285	282	291	292	302	315	311	315	322	330	350	361	382	395	410
303	243	252	255	257	257	265	270	275	271	282	276	287	280	286	290	289	306	319	331	336	347	360
304	259	262	270	264	269	270	277	276	283	280	286	288	293	294	298	318	322	327	346	356	366	389
305	226	239	238	238	248	248	254	258	266	266	279	278	289	290	301	308	313	317	337	348	371	375
306	245	246	256	254	264	271	272	274	284	286	295	299	304	306	316	321	335	354	365	378	396	411
307	223	233	233	238	242	246	247	254	255	263	267	278	283	288	287	298	306	321	325	342	346	365
308	252	258	262	266	263	270	271	276	279	287	280	291	294	303	303	307	329	341	359	370	382	397
3010	219	221	232	234	237	241	244	244	246	251	256	259	266	272	271	271	284	291	303	312	322	332
PR																						
302	237	238	243	240	243	243	246	253	252	251	262	256	267	273	280	276	288	293	309	315	313	327
303	237	239	245	247	249	250	257	258	262	265	261	266	272	274	282	291	287	299	301	308	318	329
304	228	229	239	237	237	241	240	248	249	256	259	263	263	263	261	271	273	292	300	311	319	322
305	221	225	224	230	228	230	231	238	239	241	247	250	252	257	266	273	280	289	299	310	307	330
306	241	249	254	257	256	259	257	261	264	274	268	275	264	272	272	285	297	306	316	325	330	338
307	233	233	236	234	241	249	253	251	246	246	252	253	258	260	265	273	279	290	297	311	323	331
308	237	233	241	248	251	257	259	261	261	267	274	269	279	277	278	282	295	310	320	329	338	348
3010	222	231	235	232	240	241	244	246	247	249	250	257	262	270	272	272	278	287	300	311	327	342
NPR																						
302	227	227	228	228	229	230	228	230	230	227	224	228	227	234	233	230	233	234	240	241	242	245
303	229	229	234	230	230	224	230	232	235	228	235	237	240	234	236	232	241	242	244	241	246	245
304	232	239	239	245	245	246	237	246	247	249	246	246	245	247	245	252	251	248	255	253	256	251
305	241	234	238	239	243	244	246	242	248	249	250	244	245	250	255	254	248	254	255	259	256	253
306	246	242	248	249	250	244	245	250	255	254	248	254	255	255	260	258	260	263	265	261	257	254
307	235	237	239	241	238	241	237	239	237	236	235	239	240	240	239	239	240	239	240	248	254	257
308	248	256	261	267	265	262	264	267	265	263	260	263	261	255	257	259	267	264	266	270	269	263
309	213	216	214	217	208	212	226	218	215	217	218	223	216	225	223	226	219	232	229	228	233	223

Table 16. NEONATAL DATA FOR SEV EXPERIMENT.

RAT ID	NUMBER PER LITTER	AVERAGE PUP WEIGHT (g)	TOTAL LITTER WEIGHT (g)
PC			
302	13	6.9 (0.4)	90.3
303	9	6.9 (0.4)	62.1
304	10	7.6 (0.4)	75.9
305	14	5.7 (0.3)	79.3
306	15	6.8 (0.5)	101.7
307	11	7.3 (0.5)	80.5
308	13	6.7 (0.4)	86.6
3010	8	6.1 (0.4)	49.1
PR			
302	11	5.7 (0.4)	62.9
303	8	6.2 (0.4)	49.8
304	9	7.5 (0.3)	67.6
305	11	7.2 (0.4)	79.4
306	9	7.2 (0.3)	65.2
307	7	7.5 (0.2)	52.3
308	10	7.1 (0.4)	70.9
3010	12	6.6 (0.4)	78.7

Table 17. AVERAGE NEONATAL ORGAN WEIGHTS PER LITTER FOR SEV EXPERIMENT (expressed in grams).

RAT ID	BRAIN WEIGHT	HEART WEIGHT	LIVER WEIGHT	KIDNEY WEIGHT	LUNG WEIGHT
PC					
302	0.255 (0.008)	0.033 (0.003)	0.353 (0.030)	0.058 (0.003)	0.113 (0.005)
303	0.269 (0.011)	0.030 (0.001)	0.319 (0.011)	0.064 (0.002)	0.110 (0.007)
304	0.284 (0.006)	0.038 (0.003)	0.375 (0.145)	0.074 (0.002)	0.132 (0.005)
305	0.193 (0.008)	0.023 (0.002)	0.361 (0.028)	0.044 (0.001)	0.093 (0.010)
306	0.279 (0.005)	0.035 (0.001)	0.328 (0.009)	0.071 (0.003)	0.118 (0.010)
307	0.274 (0.009)	0.037 (0.004)	0.372 (0.042)	0.068 (0.007)	0.125 (0.014)
308	0.271 (0.016)	0.032 (0.002)	0.350 (0.015)	0.069 (0.006)	0.121 (0.012)
3010	0.236 (0.005)	0.029 (0.004)	0.297 (0.028)	0.055 (0.005)	0.116 (0.010)
PR					
302	0.222 (0.013)	0.028 (0.003)	0.281 (0.003)	0.048 (0.004)	0.090 (0.005)
303	0.228 (0.009)	0.024 (0.002)	0.365 (0.014)	0.054 (0.006)	0.097 (0.007)
304	0.285 (0.004)	0.039 (0.003)	0.340 (0.014)	0.077 (0.003)	0.133 (0.006)
305	0.286 (0.011)	0.037 (0.003)	0.365 (0.024)	0.069 (0.002)	0.116 (0.011)
306	0.280 (0.007)	0.036 (0.001)	0.338 (0.018)	0.073 (0.003)	0.117 (0.006)
307	0.278 (0.013)	0.036 (0.003)	0.333 (0.004)	0.072 (0.002)	0.120 (0.006)
308	0.282 (0.010)	0.034 (0.002)	0.330 (0.013)	0.072 (0.001)	0.117 (0.005)
3010	0.245 (0.012)	0.034 (0.004)	0.335 (0.018)	0.058 (0.008)	0.122 (0.004)

Table 18. NEONATAL SKELETAL MUSCLE ANALYSIS FOR GASTROCNEMIUS MUSCLE IN PC SEV GROUP

Group	Fiber # Analysed	# Nuclei per Muscle Fiber	Nuclear Position (central)	Muscle Fiber Diameter	Ratio Muscle to C.T. %	ATPase	NADH
PC301A	40	2	0	3.4 (.84)	63	ND	ND
PC302A	37	2	4	3.1 (.70)	72	ND	ND
PC302B	42	2	7	3.4 (.76)	78	ND	ND
PC302C	28	2	2	3.6 (.74)	52	ND	ND
PC303D	47	2	1	3.2 (.73)	70	ND	ND
PC306C	68	2	1	3.1 (.76)	67	ND	ND
PC306D	49	2	2	3.3 (.88)	56	ND	ND
PC307C	52	2	3	3.2 (.83)	50	ND	ND
PC307D	45	2	10	3.1 (1.0)	54	ND	ND
PC307E	37	2	2	3.0 (.67)	62	ND	ND
PC308D	39	2	0	2.9 (.50)	64	ND	ND
PC309B	52	2	0	3.0 (.81)	44	ND	ND
PC309C	42	2	0	3.0 (.56)	49	ND	ND
PC309E	58	2	2	3.1 (.72)	63	ND	ND
PC3010A	40	2	3	3.3 (.78)	38	ND	ND
PC3010B	40	2	0	2.8 (.59)	53	ND	ND
PC3010C	51	2	1	3.3 (.75)	59	ND	ND
PC3010E	53	2	3	2.7 (.57)	54	ND	ND
N=18	45.556 (9.256)	2	2.278 (2.63)	3.127 (0.2)	58.2 (10.2)		

Table 19. NEONATAL SKELETAL MUSCLE ANALYSIS FOR DIAPHRAGM MUSCLE IN PR SEV GROUP

Group	Fiber # Analysed	# Nuclei per Muscle Fiber	Nuclear Position (central)	Muscle Fiber Diameter	Ratio Muscle to C.T. %	ATPase	NADH
PR302B	39	2	0	4.3 (.97)	51	D	ND
PR302D	41	2	0	4.6 (.95)	65	D	ND
PR302E	37	2	0	4.3 (.88)	59	D	ND
PR304A	28	2	1	4.5 (1.0)	49	D	ND
PR304D	46	2	1	4.6 (.83)	73	D	ND
PR305B	33	2	0	4.8 (.77)	51	D	ND
PR306A	35	2	0	4.5 (.89)	62	D	ND
PR306C	37	2	0	4.5 (.77)	64	D	ND
PC308B	38	2	0	4.7 (1.0)	61	D	ND
PR308D	37	2	0	4.5 (.87)	64	D	ND
PR3010A	40	2	0	4.5 (1.1)	56	D	ND
PR3010D	37	2	0	4.5 (.87)	61	D	ND
PR3010E	33	2	0	4.5 (1.0)	67	D	ND
N=13	37.0 (4.359)	2		4.528 (0.14)	60.23 (6.95)		

Table 20. NEONATAL SKELETAL MUSCLE ANALYSIS FOR DIAPHRAGM MUSCLE IN PC SEV GROUP

Group	Fiber # Analysed	# Nuclei per Muscle Fiber	Nuclear Position (central)	Muscle Fiber Diameter	Ratio Muscle to C.T. %	ATPase	NADH
PC301A	35	2	0	4.4 (.91)	67	D	ND
PC303B	33	2	0	4.7 (.73)	56	D	ND
PC303C	37	2	0	4.5 (.80)	54	D	ND
PC304D	31	2	0	4.7 (.68)	56	D	ND
PC304E	36	2	1	4.5 (.81)	59	D	ND
PC307A	39	2	0	4.3 (.87)	52	D	ND
PC30CB	41	2	0	4.4 (.87)	63	D	ND
PC308B	39	2	0	4.4 (.81)	55	D	ND
N=9	36.56 (3.17)	2		4.51 (.156)	56.67 (5.7)		

Table 21. NEONATAL SKELETAL MUSCLE ANALYSIS FOR STERNOMASTOID MUSCLE IN PR SEV GROUP

Group	Fiber # Analysed	# Nuclei per Muscle Fiber	Nuclear Position (central)	Muscle Fiber Diameter	Ratio Muscle to C.T. %	ATPase	NADH
PR302A	41	2	4	3.9 (.91)	53	ND	ND
PR302E	44	2	1	3.8 (1.1)	61	ND	ND
PR304E	35	2	0	4.0 (.78)	47	ND	ND
PR305B	42	2	0	3.8 (.67)	54	ND	ND
PR306A	47	2	0	3.7 (.73)	57	ND	ND
PR306B	44	2	0	4.0 (.82)	55	ND	ND
PR306D	47	2	4	3.7 (.92)	49	ND	ND
PR306E	45	2	3	3.6 (.77)	40	ND	ND
PC307D	43	2	1	3.7 (.81)	41	ND	ND
PR308B	56	2	5	3.9 (1.1)	58	ND	ND
PR308C	43	2	2	4.0 (.84)	54	ND	ND
PR3010A	58	2	3	3.6 (.94)	55	ND	ND
PR3010B	41	2	8	4.1 (.98)	71	ND	ND
PR3010C	48	2	3	3.8 (.91)	70	ND	ND
PC3010D	44	2	4	3.8 (.77)	61	ND	ND
PC3010E	50	2	5	3.7 (.97)	52	ND	ND
N=16	45.5 (5.657)	2	5.825 (5.23)	3.823 (0.15)	54.875 (8.57)		

Table 22. NEONATAL SKELETAL MUSCLE ANALYSIS FOR STERNOMASTOID MUSCLE IN PC SEV GROUP

Group	Fiber # Analysed	# Nuclei per Muscle Fiber	Nuclear Position (central)	Muscle Fiber Diameter	Ratio Muscle to C.T. %	ATPase	NADH
PC302B	37	2	1	3.8 (1.1)	50	ND	ND
PC302C	44	2	0	3.7 (.83)	54	ND	ND
PC304B	40	2	0	3.6 (.81)	45	ND	ND
PC304E	40	2	2	4.2 (.79)	40	ND	ND
PC306D	45	2	1	3.8 (.81)	64	ND	ND
PC307A	44	2	4	3.5 (.76)	54	ND	ND
PC307B	43	2	2	3.6 (.70)	61	ND	ND
PC307C	49	2	1	3.6 (.94)	63	ND	ND
PC307D	42	2	4	3.8 (.92)	57	ND	ND
PC307E	54	2	6	3.4 (.94)	56	ND	ND
PC308E	47	2	2	3.6 (.90)	55	ND	ND
PC3010A	39	2	3	3.5 (.79)	40	ND	ND
PC3010B	46	2	2	3.9 (1.0)	58	ND	ND
PC3010C	44	2	1	3.8 (.89)	53	ND	ND
PC3010E	49	2	5	3.8 (.88)	60	ND	ND
N=15	44.2 (4.43)	2	5.007 (3.675)	3.697 (0.20)	54.0 (7.49)		

Table 23. NEONATAL SKELETAL MUSCLE ANALYSIS FOR GASTROCNEMIUS MUSCLE IN PR SEV GROUP

Group	Fiber # Analysed	# Nuclei per Muscle Fiber	Nuclear Position (central)	Muscle Fiber Diameter	Ratio Muscle to C.T. %	ATPase	NADH
PR302C	39	2	2	3.1 (.75)	56	ND	ND
PR302D	44	2	1	3.3 (.90)	70	ND	ND
PR302E	42	2	11	3.3 (.78)	64	ND	ND
PR305B	76	2	2	2.9 (.72)	57	ND	ND
PR307D	38	2	1	3.3 (.75)	55	ND	ND
PR308B	58	2	6	3.2 (.93)	60	ND	ND
PR308C	54	2	1	3.0 (.69)	72	ND	ND
PR308E	49	2	0	3.6 (.61)	49	ND	ND
PR3010A	44	2	2	3.4 (.87)	42	ND	ND
PR3010B	72	2	4	3.0 (.66)	70	ND	ND
PR3010C	58	2	1	3.3 (.88)	58	ND	ND
PR3010D	58	2	1	3.1 (.68)	55	ND	ND
PR3010E	50	2	6	3.0 (.83)	61	ND	ND
N=13	52.462 (11.87)	2	2.846 (3.16)	3.183 (0.19)	59.2 (8.54)		

Table 24. POSTPARTAL BODY COMPONENT ANALYSIS FOR SEV EXPERIMENT (expressed in grams).

Group	PP Weight Dead	Skin Weight	Remainder Weight
PC302	299.0	58.5	240.3
PC303	273.6	52.7	219.2
PC304	287.2	54.4	231.3
PC306	287.8	51.2	233.2
PC307	266.6	41.5	223.8
PC308	295.86	64.0	230.3
PC3010	268.6	52.5	214.3
PR302	257.0	38.9	216.5
PR304	257.8	45.4	210.4
PR305	232.0	38.0	192.7
PR306	255.8	41.3	211.3
PR307	268.0	52.2	211.3
PR308	267.5	39.8	226.2
PR3010	265.0	45.8	214.2
NPR302	241.1	32.4	207.4
NPR303	244.0	35.8	206.4
NPR304	257.2	35.0	213.8
NPR305	260.1	37.7	220.9
NPR306	260.1	31.7	220.9
NPR307	250.5	37.1	220.9
NPR308	274.5	43.5	229.3
NPR309	240.0	33.5	204.8

Table 25. DAY 22 RATIO OF SKIN WEIGHT AND CARCASS REMAINDER WEIGHT (MEAN (SD)) FOR PC-SEV, PR-SEV and NPR-SEV.

RAT ID	% SKIN WEIGHT	% REMAINDER WEIGHT	DIFF IN % BLOOD LOSS etc.
PC 302	19.6	80.3	0.1
303	19.3	80.1	0.6
304	18.9	80.5	0.6
306	17.8	81.0	1.2
307	15.6	83.9	0.5
308	21.6	77.8	0.6
3010	19.5	79.8	0.7
$\overline{X}$(SD)	18.9 (1.8)	80.5 (1.8)	0.61 (.3)
PR 302	15.1	84.2	0.7
304	17.6	81.6	0.8
305	16.4	83.1	0.5
306	16.1	82.6	1.3
307	19.5	78.8	1.7
308	14.9	84.6	0.5
3010	17.3	80.8	1.9
$\overline{X}$(SD)	16.7 (1.6)	82.2 (2.0)	1.1 (.6)
NPR 302	13.4	86.0	0.6
303	14.7	84.6	0.7
304	14.0	85.5	0.5
305	15.9	83.1	1.0
306	14.5	84.9	0.6
307	14.8	84.6	0.6
308	15.8	83.5	0.7
309	14.0	85.3	0.7
$\overline{X}$(SD)	14.6 (0.9)	84.7 (1.0)	0.68 (.1)